TOXICOLOGIST'S REVIEW

BLA: 99-1492
SPONSOR: Amgen, Inc.
PRODUCT: Novel Erythropoiesis Stimulating Protein; NM321; NESP; ARANESP™
FORMULATION/CHEMISTRY: Produced in a mammalian (CHO cells) expression system in a hyper-glycosylated form, with a MW of 37.1 kD. The glycoprotein is composed of 165 amino acids. The protein is the hyperglycosylated recombinant human EPO analog. NESP differs from native or recombinant EPO in the form of two additional N-linked oligosaccharide chains. The protein is formulated as a sterile, colorless, preservative-free solution.
PROPOSED INDICATION: Treatment of anemia associated with chronic renal failure [CRF]

ABBREVIATIONS: recombinant human erythropoietin = EPO; Novel Erythropoiesis Stimulating Protein = NESP; peripheral blood progenitor cell = PBPC; subcutaneous = SC; intravenous = IV; platelet = PLT; red blood cell = RBC; bone marrow = BM; hemoglobin = HGB; chronic renal failure = CRF

received 1/12/00; completed 8/23/00; revised 7/24/03

CROSS-REFERENCES: IND [redacted]

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INTRODUCTION:
NESP is a well-characterized recombinant protein, which is an analog of recombinant human erythropoietin (EPO). NESP stimulates erythropoiesis in a manner identical to endogenous human EPO, as well as recombinant human EPO - which is approved for use in the Rx of anemia in patients with chronic renal failure (CRF). NESP binds to cell surface receptors that are specific for EPO, resulting in the promotion of survival, proliferation, & differentiation of cells of the erythroid

lineage. The in vitro potency of NESP is lower than that of EPO due to an increase in [redacted] resulting from the addition of [redacted] containing carbohydrate & consequently weaker receptor binding. However, due to the addition of this carbohydrate, the in vivo activity of NESP is increased relative to EPO because of its longer serum half-life [~3-fold longer].

Amgen's NESP, consisting of 165 amino acids, is produced by CHO cells in which a gene has been inserted for [redacted] The protein is hyperglycosylated & has a theoretical MW of 37,100 daltons, based on the amino acid sequence & nominal carbohydrate structure. The protein contains [redacted] cysteines which form [redacted] intramolecular disulfide bonds. NESP is different from natural & recombinant EPO by [redacted] acids. This difference allows for new sequences for N-linked glycosylation.

The proposed clinical indication [per the package insert] for NESP is for the treatment of anemia associated with CRF, as NESP has been shown to stimulate erythropoiesis in this patient population. This treatment has been correlated with a reduction in RBC transfusions and improved quality of life.

The proposed package insert submitted by the sponsor states that once weekly IV or SC injections of NESP, and in some cases, SC injections once every two weeks, have resulted in efficacious results in patients. The PI-recommended starting dose of NESP for the specified patient population is 0.45 µg/kg/dose, qiw, IV/SC. The dose of NESP for each patient is variable, thus should be adjusted as clinically indicated to achieve the target HGB level. HGB levels should be determined weekly, until HGB levels stabilize, then monthly, in order to be able to titrate the dose of NESP. The PI states that dose adjustments should not occur sooner than one month apart. If the HGB is increasing to the point of approaching the preset upper limit, the NESP dose should be reduced by 25%. If the HGB continues to increase, NESP should be discontinued until the HGB level begins to trend downward, at which point dosing can be resumed at a 25% reduction. Additional instructions are provided in the PI, including instructions regarding maintenance dosing [again, which are patient-dependent].

Formulation - ARANESP™ will be distributed in [redacted] & vials & is manufactured in two formulations: either a polysorbate [free of serum-derived albumin] or albumin [human] formulation. [redacted] The vial doses range from 15-500 µg & are filled to a withdrawable volume of 1.0 mL. NESP is

formulated in a sterile, preservative-free solution, containing: **[Polysorbate soln]** 2.12 mg sodium phosphate monobasic monohydrate, 0.66 mg sodium phosphate dibasic anhydrous, 0.05 mg polysorbate 80, 8.18 mg sodium chloride, & Water for Injection USP, pH 6.2 ±0.2; and **[Albumin soln]** 2.5 mg human albumin, 2.23 mg sodium phosphate monobasic monohydrate, 0.53 mg sodium phosphate dibasic anhydrous, 8.18 mg sodium chloride, & Water for Injection USP, pH 6.0 ±0.3.

Unless specified, all preclinical studies were performed using the NESP formulation containing HSA.

Preclinical Pharmacology Studies

In vitro

1. Effect of Carbohydrate Binding of rHuEPO & NESP to the Human EPO Receptor; report #PD001; performed at Amgen; lot [redacted] NM321; 6/99; part III of V, vol 39

2. Studies of the In Vitro Biological Activity of NESP; report #PD002; performed at Amgen; 3/99; lot [redacted] part III of V, vol 39

3. Comparison of NESP & rHuEPO in the [redacted] Assay; report #PD013; performed at Amgen; lot [redacted] 6/99; part III of V, vol 39

In vivo

1. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD003; performed at Amgen; lot [redacted] 10/93; part III of V, vol 39

2. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD004; performed at Amgen; lot [redacted] 4/94; part III of V, vol 39

3. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD005; performed at Amgen; lot [redacted] 8/94; part III of V, vol 40

4. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD006; performed at Amgen; lot [redacted] 10/95; part III of V, vol 40

5. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD007; performed at Amgen; lot [redacted] 11/95; part III of V, vol 40

6. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD008; performed at Amgen; lot [redacted] [GLP lot - also used in toxicology studies]; 4/96; part III of V, vol 40

7. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD009; performed at Amgen; lot [redacted] 7/96; part III of V, vol 40

8. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD010; performed at Amgen; lot [redacted] 3/97; part III of V, vol 40

9. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice; report #PD011; performed at Amgen; lot [redacted] [GMP lot]; 8/97; part III of V, vol 40

10. Relative Potency of NESP & rHuEPO; report #PD014; performed at Amgen; lot [redacted] 10/93 - 8/97; part III of V, vol 40

11. Immunogenicity of NESP & rHuEPO; report #PD012; performed at Amgen; lot [redacted] 10/93 - 8/97; part III of V, vol 40

Pharmacology Studies

In vitro

1. Effect of Carbohydrate Binding of rHuEPO & NESP to the Human EPO Receptor

NESP differs from rHuEPO at [redacted] amino acid positions, having two new carbohydrate chains [redacted] more than rHuEPO. The receptor binding activity & the in vitro activity of NESP is lower compared to rHuEPO. Following exposure of NESP to [redacted], a reduction in the number of [redacted] to the carbohydrate was noted, with no alteration in the peptide backbone. Treatment of NESP with [redacted] resulted in increased in vitro activity & treatment of both NESP & rHuEPO resulted in comparable receptor binding activities.

2. Studies of the In Vitro Biological Activity of NESP

[redacted] cells cultured with NESP [redacted] ng/mL) + [redacted] resulted in stimulation of [redacted] [the earliest hematopoietic progenitor cell of the myeloid lineage]. The minimum biologic dose of NESP was 1 ng/mL & optimal levels of [redacted] were reached

at 250 ng/mL of NESP. The EC_{50} of NESP = 21 ng/mL. The EC_{50} of EPO was 2.1 ng/mL; the minimal biologic dose was 0.1 ng/mL, & the optimal concentration was 100 ng/mL.

[redacted] cells cultured with NESP (0.01-1000 ng/mL) + SCF + MGDF resulted in stimulation of BFU-E. The minimum biologic dose of NESP was 0.5-1 ng/mL & optimal levels of BFU-E were reached at 100-500 ng/mL of NESP. The EC_{50} of NESP = 10 ng/mL. The EC_{50} of EPO was 1.0 ng/mL & optimal concentration was 5 ng/mL.

[redacted] cells cultured with NESP (0.01-1000 ng/mL) resulted in stimulation of CFU-E [the most mature hematopoietic progenitor cells]. The minimum biologic dose of NESP was 0.3 ng/mL & the optimal levels of CFU-E were reached at 50 ng/mL of NESP. The EC_{50} of NESP = 2.0 ng/mL. The EC_{50} of EPO was 0.44 ng/mL; the minimal biologic dose was 0.03 ng/mL, & optimal concentration was ~5 ng/mL.

3. Comparison of NESP & rHuEPO in the [redacted] Assay

NESP binds to, & activates, the EPO receptor (EPOR) on hematopoietic progenitor cells. There is a progressive decrease in EPOR affinity [measured [redacted] assay using [redacted] cells] with increasing [redacted] content for NESP. Compared to EPO, NESP has a lower potency in the in vitro assays due to the increase in [redacted]-containing carbohydrate - the greater the [redacted] content, the lower the potency. About 4.3-fold more NESP was required to compete with the binding of [redacted]-EPO to the EPOR.

[redacted]

NESP stimulates all stages of erythroid progenitor cells in a dose-dependent manner is lineage-specific - i.e, erythroid cells. NESP effects erythroid progenitor proliferation & maturation, as the cells mature into RBCs.

In vivo

Studies 1-9. A Comparative Study of the Effect of NESP & rHuEPO on the HCT of Normal Mice

NESP given via IP, SC, or IV routes to normal [redacted] female mice, qiw or tiw, causes a dose-dependent increase in HCT. NESP & EPO (0.156-10 μg/kg) dosing (IV, IP, SC) in mice, tiw, for 6 weeks resulted in dose-dependent increases in HCT. NESP-induced changes in HCT were comparable across the injection routes. NESP was ~4-fold more potent than EPO when dosed tiw - based on the HCT response or on the AUC.

When given tiw, NESP has ~3-4-fold greater potency & ~20-fold greater potency when given qiw compared to EPO [due to the 3-fold longer serum half-life]. Higher [redacted] containing isoforms of NESP ([redacted] residues/molecule) have higher in vivo biological activity compared to EPO ([redacted] residues/molecule).

Normal mice were IP or IV dosed, qiw, with NESP (3.75-22.5 μg/kg) or EPO (7.5-30 μg/kg) for 6 weeks. Via the IP route, the HCT response obtained at 30 μg/kg EPO was less than the response obtained with 3.75 μg/kg NESP. Via the IV route, the HCT response obtained at 200 μg/kg EPO was comparable to the response obtained with 6.25 μg/kg NESP.

A 4-fold higher amount of NESP is needed when dosing qiw compared to tiw in order to achieve the same biological effect in mice. NESP is as effective when given qiw, as when given tiw.

10. Relative Potency of NESP & rHuEPO

This report summarizes the series of experiments conducted from 10/93 to 9/97 in mice, which compared the effect of injections of NESP vs. rHuEPO on HCT levels in mice [studies #1-9 of this section]. Refer to the previously written summarization of these studies (above) for review.

11. Immunogenicity of NESP & rHuEPO

Normal & [redacted] mice SC injected on days 0, 7, 14, 21, & 28 with 0.3125, 0.625, 1.25, or 2.5 μg/kg/dose of NESP or rHuEPO, displayed dose-dependent increases in the number of mice that developed Abs. The Ab titers, as well as the presence of a neutralizing response, were similar for both products.

Comment:

- Both EPO & NESP are biologically active in mice, but as they share only [redacted] homology between amino acids, Abs to either material were measured in all experiments. The SC route was the most immunogenic route & the IV route, the least immunogenic. Very few humans have exhibited anti-EPO Abs. Since NESP is a hyperglycosylated analog of EPO with a longer half-life, NESP may be more immunogenic in humans.

PK/ADME Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Dose-Ranging PK of IV Single Dose NM321 in [redacted] Mice; study #PK96086; performed at Amgen (non-GLP); lot [redacted] 5/99; part III of V, vol 43

2. Single Dose PK of NM321 in Rats: Parallel Group IV/SC; study #PK96029; performed at Amgen (non-GLP); lot [redacted] 9/97; part III of V, vol 43

3. PK of NM321 Over 26 Days by a Single Dose SC and IV Delivery in the [redacted] Dog; study #PK96031; performed at Amgen (non-GLP); lot [redacted] 9/97; part III of V, vol 44

4. PK of [redacted]-rEPO & [redacted]-NM321 in the Dog Following a Single IV Dose; study #6271-119; performed at [redacted] (non-GLP); lot [redacted] 10/97; part III of V, vol 44

5. PK from "Multiple Dose PK of NM321 Following SC Delivery in the [redacted] Dog"; study #PK96032; performed at Amgen (non-GLP); lot [redacted] 8/98; part III of V, vol 44

6. Distribution & Excretion of [redacted]-NESP in [redacted] Rats Following a Single SC/IV Administration; study #PK100215; performed at Oread (per GLP); lot [redacted] 1/99; part III of V, vol 45

7. A PK Study of NM321 in Sham, Monolaterally Nephrectomized & Bilaterally Nephrectomized Rats; study #PK97090; performed at [redacted] (non-GLP); lot [redacted] 8/98; part III of V, vol 46

8. Determination of (1) Linear Dose Range & (2) Hepatic Elimination of [redacted] NESP in Rats Using the Accelerated Infusion Technique; study #100133; performed at Amgen (non-GLP); lot [redacted] 8/98; part III of V, vol 46

9. Comparison of the PK of NESP and [redacted] NESP Following IV Bolus Administration in the Presence & Absence of Galactose-Receptor Competitor in Male [redacted] Rats; study #100134; performed at Amgen (non-GLP); lot [redacted] (des-NESP), [redacted] (NESP); 12/98; part III of V, vol 46

10. Pilot Study for the Comparison of PK Following IV & SC Dosing of HSA-Containing & HSA-Free NESP in [redacted] Dogs; study #100388; performed at [redacted] (non-GLP); lot [redacted] HSA-free), [redacted] (HSA); 4/99; part III of V, vol 47

11. Determination of the Effect of Formulation Concentration on the PK of NESP Upon SC Dosing in [redacted] Dogs; study #100523; performed at [redacted] (non-GLP); lot [redacted] (HSA), [redacted] (HSA); 9/99; part III of V, vol 47

12. PK of NESP, [redacted] NESP, & NESP [redacted] Following a Single IV Bolus to Male [redacted] Rats; study #100507; performed at [redacted] (non-GLP); lot [redacted] (NESP), [redacted] (Sub-NESP), [redacted] (NESP-[redacted]; 9/99; part III of V, vol 48

PK/ADME Studies

1. Dose-Ranging PK of IV Single Dose NM321 in [redacted] Mice

Species: [redacted] mice (42 males/group)

Dose Level: 1, 10, 100 µg/kg

Route/Duration: IV/single dose

Methods: NESP was IV injected (bolus dose), & blood collected (via cardiac puncture) at 0, 5, 15, 30, 45 mins; 1, 2, 4, 8, 12, 24, 48, 72, 96 hrs, and the sera analyzed via [redacted] using a [redacted] for EPO (limit of detection = 0.16 ng/mL)

Findings: PK data are presented below:

Table 6: Non-Compartmental Parameters[a, b] Following IV Administration of NM321 to [redacted] Mice

Parameter	Units	Group 1 (1 µg/kg)	Group 2 (10 µg/kg)	Group 3 (100 µg/kg)
Concentration at time zero (C_0)	ng/mL	22.1	493	3910
Initial volume of distribution (V_0)	mL/kg	45.2	20.3	25.6
Area under the serum concentration time curve from zero to last time-point ($AUC_{(0-t)}$)	ng hr/mL	231	3100	36200
Area under the serum concentration time curve from zero to infinity ($AUC_{(0-\infty)}$)	ng hr/mL	233	3110	36300
Dose-normalized ($AUC_{(0-\infty)}$) ($nAUC_{(0-\infty)}$)	ng hr/mL/(µg/kg)	0.233	0.311	0.363
Terminal half-live (λ_z)	hr^{-1}	0.0720	0.0611	0.0574
Terminal half-life ($t_{1/2,z}$)	hr	9.63	11.4	12.1
Volume of distribution at steady state (V_{ss})	mL/kg	68.2	48.7	45.0
Volume of distribution associated with terminal phase (V_z)	mL/kg	59.7	52.7	48.0
Clearance (CL)	mL/hr/kg	4.30	3.22	2.75

[a] Estimated using naïve-averaged data.
[b] Numbers are rounded to three significant figures

The elimination half-life was 11.9 hrs. The PK profile was dose-linear.

2. Single Dose PK of NM321 in Rats: Parallel Group IV/SC
Species: SD rats (2/timepoint)
Dose Levels: 1, 3, 10, 30, 100 µg/kg
Route/Duration: IV/SC injection/single dose

Methods: An [redacted] was used, with a limit of quantification of [redacted] Both compartmental & non-compartmental analyses were performed.

Findings:
For both routes the PK profile is dose-linear & elimination rate limited. The material is cleared slowly from the circulation (~5.4 mL/hr/kg). Terminal half life is ~17 hrs. Bioavailability via SC injection is ~41-54%:

Table 2: Summary Non-Compartmental Parameters - Single Intravenous Dose (1, 3, 10, 30 and 100 µg/kg)*

Dose µg/kg	$AUC_{(0-\infty)}$ ng hr/mL	C_{max} ng/mL	CL mL/hr/kg	V_z mL/kg	$t_{1/2\lambda z}$ hr
1	189	21.7	5.30	106	19.8
3	575	68.2	5.25	83.1	18.3
10	1920	223	5.25	101	15.7
30	6280	708	4.80	88.5	16.4
100	17900	2300	5.60	104	15.8

*Mean of 2 individuals; observed values used

Table 3: Summary Non-Compartmental Parameters - Single Subcutaneous Dose (1, 3, 10, 30 and 100 µg/kg)*

Dose µg/kg	$AUC_{(0-\infty)}$ ng hr/mL	C_{max} ng/mL	T_{max} hr	CL/F mL/hr/kg	V_z/F mL/kg	$t_{1/2\lambda z}$ hr	F* %
1	88.7	1.90	18	11.5	324	19.6	47
3	256	5.17	18	11.9	278	16.6	45
10	797	15.8	24	12.6	309	17.0	41
30	2620	51.9	12	11.5	276	16.7	42
100	9670	183	24	10.4	264	17.8	54

*Mean of 2 individuals; observed values used
*Bioavailability calculated from: $AUC_{0-\infty,SC} / AUC_{0-\infty,IV}$ (mean values)

3. PK of NM321 Over 26 Days by a Single Dose SC and IV Delivery in the [redacted] Dog
Species: [redacted] dogs (1/sex/grp)
Dose Levels: 0, 1, 3, 10, 30 µg/kg
Route/Duration: IV/SC injection/single dose

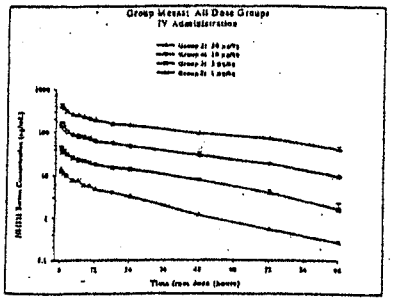
Methods: An [redacted] was used, with a limit of quantification of [redacted] ng/mL. Both [redacted] analyses were performed.

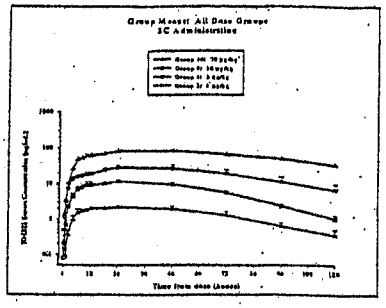
Findings:
IV - NM321 had linear & saturable clearance pathways. Terminal half life ranged from 20.2-41.2 hrs. Clearance decreased with increasing dose levels & there was a progressive increase in elimination half-life with increasing dose. The PK profile was not affected by sex.

SC - Bioavailability was ~65-78%

Group:	2	3	4	5	7	8	9	10
Compound:	NM321	NM321	NM321	NM321	NM321	NM321	NM321	NM321
Dose (µg/kg):	1	3	10	30	1	3	10	30
Route:	IV	IV	IV	IV	SC	SC	SC	SC
Results:								
Parameter (units):								
T_{max} (hr)	----	----	----	----	24	24	24	12.5
C_{max} (ng/mL)	14.7	49.0	173	416	2.13	11.7	28.8	113
AUC_{0-inf} (ng*hr/mL)	224	1010	3910	13300	174	787	2530	8680
$t_{1/2}$ (hr)	20.2	23.1	32.0	41.2	25.0	19.5	30.1	43.5
CL (mL/hr/kg)*	4.45	3.00	2.55	2.30	5.85	3.80	4.15	3.50
$AUC_{0-inf,SC}$ / $AUC_{0-inf,IV}$	----	----	----	----	0.78	0.78	0.65	0.65

*CL/F calculated for SC administration





4. PK of [redacted]-rEPO & [redacted]-NM321 in the Dog Following a Single IV Dose

Species: [redacted] dogs (2 females/grp)

Dose Levels: ~6 x 10^6 [redacted] [0.04 µg/kg]

Route/Duration: IV injection/single dose

Methods: Following IV injection, blood was obtained at 5, 15, & 30 mins, and at 1, 2, 5, 8, 12, 16, & 24 hrs postdose, then analyzed for [redacted] Blood was collected predose & on day 7 for hematology.

Findings: The T_{max} was at 0.5 mins, steadily declining by 24 hrs. A biphasic serum concentration time profile was noted for both materials:

$t_{½\alpha}$ = 0.34 hrs (NESP)	$t_{½\beta}$ = 25.0 hrs (NESP)
$t_{½\alpha}$ = 0.40 hrs (EPO)	$t_{½\beta}$ = 7.2 hrs (EPO)
V_D = 55.9 mL/kg (NESP)	CL = 21.5 mL/kg/hr (NESP)
V_D = 60.8 mL/kg (EPO)	CL = 69.2 mL/kg/hr (EPO)

5. PK from "Multiple Dose PK of NM321 Following SC Delivery in the [redacted] Dog"

Species: [redacted] dogs (1/sex/grp)

Dose Levels: 0, 1, 3, 10 µg/kg

Route/Duration: SC injection/dose days = 0, 2, 5, 7, 9, 12

Methods: Following SC injection, blood was obtained at various time intervals post-each dose, followed by analysis via an [redacted] [limit of quantification = [redacted] ng/mL]. Blood was collected out to day 46 for hematology. Abs were measured on days 1 & 12.

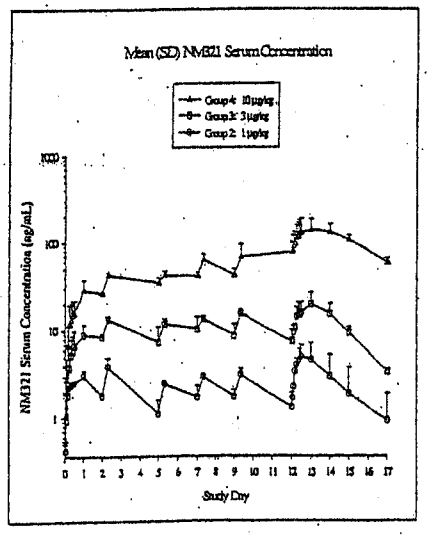
Findings: The day 12 sera levels of the 10 µg/kg grp were ~2-fold higher than expected based on earlier studies performed.

↑ HCT, HGB, RBCs, retics, with the change in retics being the most sensitive indicator of NESP activity, with peak levels on day 9/12 - ~10-200-fold increase from baseline

By day 12, 3/8 dogs were seropositive [one control & two Rx dogs]

Group:	2		3		4	
Compound:	NM321		NM321		NM321	
Dose (µg/kg):	1		3		10	
Route:	SC		SC		SC	
Results (Mean):						
Parameter (units):	Day 0	Day 12	Day 0	Day 12	Day 0	Day 12
T_{max} (hr)	24.0	16.0	36.0	24.0	36.0	30.0
C_{max} (ng/mL)	3.04	5.20	9.60	19.6	30.2	140
AUC_{0-t} (ng*hr/mL)	---	369	---	1510	---	18100
$t_{1/2,z}$ (hr)	---	36.9	---	34.0	---	62.8
CL/F (mL/hr/kg)*	---	3.89	---	2.05	---	0.559

*Relative Clearance



6. Distribution & Excretion of [redacted]-NESP in [redacted] Rats Following a Single SC/IV Administration

Species: [redacted] rats (2/sex/grp/timepoint)

Dose Levels: 100 µg/kg

Route/Duration: SC/IV injection + kill at each designated timepoint

Methods: Following SC/IV injection, blood was obtained at 1, 8, 24, 72, & 168 hrs postdose. Urine, feces, & cage rinse were collected every 24 hrs through 168 hrs postdose. Various tissues were collected at kill for evaluation of [redacted]

Findings:

IV - The highest level of [redacted] was in the sera at all timepoints, with ≥85% of the [redacted] associated with the protein (via precipitation of sera). Other tissues with notable [redacted] included thyroid, trachea, & stomach. A terminal half life of 20-21 hrs was noted. About 65% of the dose was excreted in the urine, most of it within 48 hrs of dosing. Recovery in the feces was 17-30% & it was 2-4% in the tissues.

SC - The highest level of [redacted] [excluding the thyroid & injection site] was in the sera at all timepoints, with ∃90% of the [redacted] associated with the protein (via precipitation of sera). A terminal half life of 24 hrs was noted. About 65% of the dose was excreted in the urine, most of it within 48 hrs of dosing. Recovery in the feces was 20-22% & it was 3-4% in the tissues. Bioavailability was ~45-53%.
There were no notable differences between males & females.

7. A PK Study of NM321 in Sham, Monolaterally Nephrectomized & Bilaterally Nephrectomized Rats

Species: [redacted] rats (5 males/grp)

Dose Levels: 0.5, 1, 5 µg/kg/dose

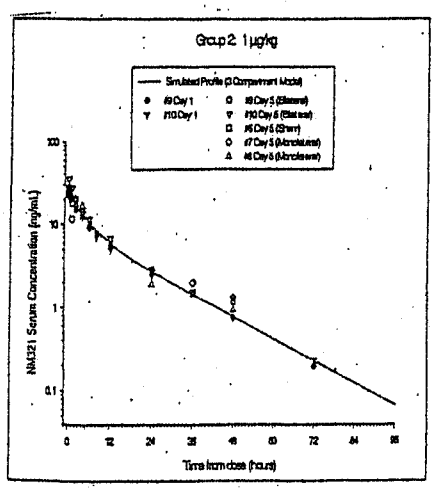
Route/Duration: IV injection on days 1 & 5 + kill

Methods: Sham, monolateral, or bilateral nephrectomies were performed on rats, followed by injection on days 1 & 5. Blood (for PK) & urine (for PK, BUN, & creatinine) samples were obtained out to 96 hrs postdose.
The samples were assayed via [redacted] with a limit of quantitation of [redacted] ng/mL (serum) & [redacted] ng/mL (urine). In addition, the sera of 3/5 rats were to be evaluated in a bioassay, however, bacterial contamination prevented use of the assay.

Findings: Although greater in variability, the PK profiles were similar for all doses for all rats - reflective of a minimal contribution of renal clearance to total clearance of NESP. Approximately 2.4% (control), 2.2% (sham), & 0.8% (nephrectomized) of the total dose was excreted via the kidneys. No overt effect on BUN & creatinine was noted for the different groups.

Group:	1			2			3		
Dose (µg/kg):	0.5			1			5		
Parameter (units)*:									
Day 1									
V_0 (mL/kg)	34.8			36.6			28.4		
CL (mL/hr/kg)	4.76			4.44			3.71		
Day 5 (Surgery)									
Nephrectomy:	Sham	Mono-lateral	Bi-lateral	Sham	Mono-lateral	Bi-lateral	Sham	Mono-lateral	Bi-lateral
V_0 (mL/kg)	34.4	38.2	32.7	34.8	36.5	36.2	35.7	17.3	24.7
CL (mL/hr/kg)	3.52	3.30	4.03	3.77	3.63	5.39	3.62	2.95	3.93

*Mean non-compartmental parameter



8. Determination of (1) Linear Dose Range & (2) Hepatic Elimination of [redacted] NESP in Rats Using the Accelerated Infusion Technique

Species: [redacted] rats (4 males/grp)

Dose Levels: 352 µg; infused at 0.55 mL/hr; increased during the 3 hrs to 0.95 mL/hr (total of 2.2 mL/rat)

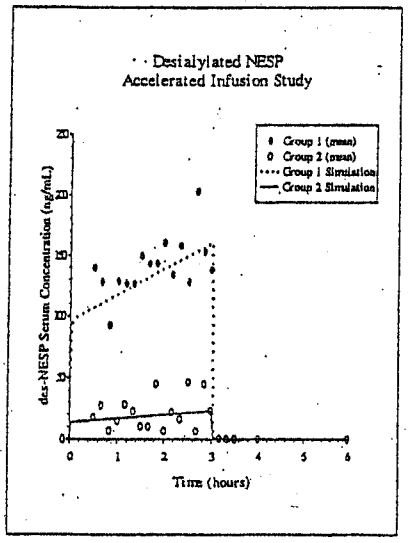
Route/Duration: single IV infusion (3 hrs)

Methods: One group had jugular & femoral veins pre-cannulated; one group had jugular & hepatic portal veins pre-cannulated

Blood samples were obtained from the jugular vein out to 33 hrs post-end-of-infusion. The samples were assayed via [redacted] with a limit of quantitation of [redacted] ng/mL.

Findings: Infusion of des-NESP via the femoral vein resulted in rapid clearance (3020 mL/hr/kg), with a terminal half-life of 1.3 mins. [unable to determine linear range]. Sera levels of des-NESP were ~7-fold higher relative to infusion via the portal vein [141 vs 21.5 ng/mL]. The AUC via femoral infusion was ~85% higher relative to hepatic vein infusion, indicating that des-NESP is extensively hepatically cleared.

Group Route Parameter	Units	Group 1 Femoral Vein	Group 2 Hepatic Portal Vein	Ratio (HPV:FV)
T_{max}	hr	2.667	2.5	0.937
C_{max}	ng/mL	203	45.9	0.226
T_{last}	hr	4	4	1
C_{last}	ng/mL	0.049	0.058	1.18
$AUC_{(0-t)}$	ng hr/mL	389	56.8	0.146
$AUC_{(0-\infty)}$	ng hr/mL	389	56.8	0.146
CL	mL/hr/kg	3020	20700	6.85
$t_{1/2,z}$	hr	0.0220	0.0310	1.41



Comment:

- [Per the sponsor] The proposed clearance mechanism of NESP is desialylation by tissue & blood sialidases, followed by hepatic removal of the [redacted]NESP [redacted]-NESP) via its galactose receptor. The intent of this study was to determine the contribution of first-pass hepatic clearance to the overall clearance of the protein.

9. Comparison of the PK of NESP and [redacted]-NESP Following IV Bolus Administration in the Presence & Absence of Galactose-Receptor Competitor in Male [redacted] Rats

Species: [redacted] rats (3 males/grp)

Dose Levels: 10 µg/kg

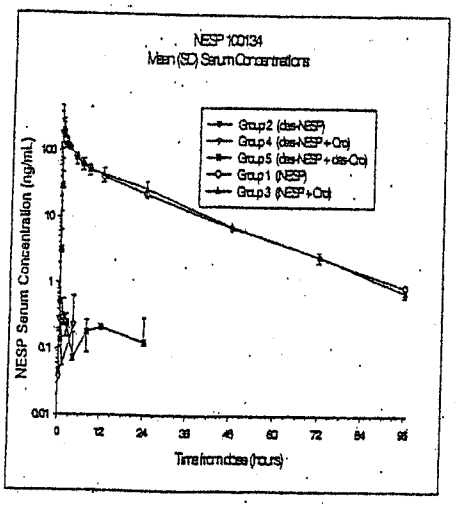
Route/Duration: single IV bolus

Methods: Rats received either NESP or [redacted] NESP with/without 1 mg/rat of intact orosomucoid (Oro) or des-Oro - a protein which is known to be cleared in a mechanism similar to NESP (via a galactose receptor). Blood samples were obtained up to 96 hrs postdose. The samples were assayed via [redacted] with a limit of quantitation of [redacted] ng/mL for NESP & [redacted] ng/mL for [redacted]-NESP.

Findings: Injection of NESP + Oro did not affect the known PK profile of NESP, with measurable sera levels only out to 5 mins. Injection of [redacted] NESP + des-Oro resulted in measurable sera levels out to 24 hrs [lower clearance] - indicative of some clearance of des-NESP via the hepatic galactose receptor.

Group		Group 1	Group 2	Group 3[a]	Group 4	Group 5
NESP form Oro form		NESP -	des-NESP -	NESP Oro	des-NESP Oro	des-NESP des-Oro
Parameter	Units					
T_{last}	hr	96.0		96.0		10.8
C_{last}	ng/mL	0.914	Unable to	0.785	Unable to	0.789
$AUC_{(0-t)}$	ng hr/mL	1800	Estimate	1910	Estimate	35.0
$AUC_{(0-\infty)}$	ng hr/mL	1820	Parameters	1930	Parameters	35.0
CL	mL/hr/kg	5.52		5.25		290
$t_{1/2,z}$	hr	15.3		14.3		0.0617

[a]For all Groups n=3, except Group 3 where n=2



10. Pilot Study for the Comparison of PK Following IV & SC Dosing of HSA-Containing & HSA-Free NESP in [redacted] Dogs

Species: [redacted] (1 male/grp)

Dose Levels: 3 μg/kg/dose

Route/Duration: IV/SC; one agent was injected on day 1, followed by the other agent on day 7 (in the same dog)

Methods: Dogs were either IV or SC injected with one agent on day 1, followed by a wash-out interval. The same dog was then injected (same route) with the other agent on day 7. Blood samples were obtained up to 96/120 hrs postdose. The samples were assayed via [redacted] with a limit of quantitation of [redacted] ng/mL.

Findings: The limited data generated from a very minimal number of dogs indicate that either SC or IV injection of HSA-free or HSA-containing NESP resulted in comparable PK profiles for that respective route.

Parameter[a]	Unit	HSA-containing		HSA-free	
		IV Dosing (3 µg/kg)			
		3111164	3117651	3111164	3117651
C_o	ng/mL	51.9	65.8	64.9	59.0
V_s	mL/kg	57.8	45.6	46.2	50.9
AUC $_{(0-t)}$	ng hr/mL	1070	1390	1310	1130
AUC $_{(0-\infty)}$	ng hr/mL	1090	1450	1360	1170
		SC Dosing (3 µg/kg)			
		3118347	3123090	3118347	3123090
T_{max}	hr	4	24	24	24
C_{max}	ng/mL	20.0	13.6	13.6	9.84
AUC $_{(0-t)}$	ng hr/mL	1000	890	948	660
AUC $_{(0-\infty)}$	ng hr/mL	1070	961	1050	789

[a] Numbers rounded to three significant figures

11. Determination of the Effect of Formulation Concentration on the PK of NESP Upon SC Dosing in [redacted] Dogs

Species: [redacted] (4 males/grp)

Dose Levels: 30 µg/kg/dose; concentrations of 100 or 500 µg/mL of each agent

Route/Duration: IV/SC; one agent was injected on day 1, followed by the other agent on day 14 (in the same dog)

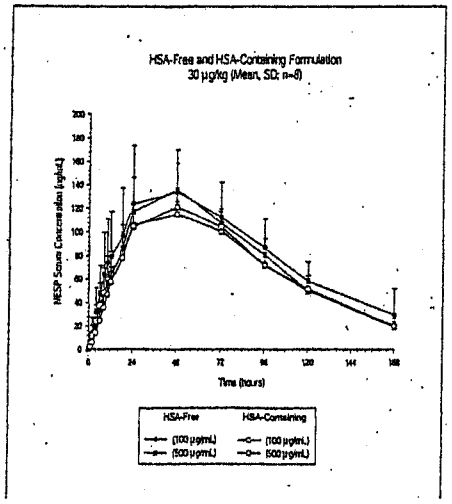
Methods: Dogs were SC injected with one concentration (either 100 or 500 µg/mL) of one agent on day 1, followed by a wash-out interval. The same dog was then SC injected with the other agent on day 14. Blood samples were obtained up to 168 hrs postdose. The samples were assayed via ELISA, with a limit of quantitation of 0.147 ng/mL.

Findings: NESP was measurable in all dogs out to 168 hrs. SC injection of NESP with or without HSA at concentrations of either 100 or 500 µg/mL resulted in comparable PK profiles.

SC Dosing (30 µg/kg)		HSA-containing[a]		HSA-free[a]	
Parameter[b]	Unit	Day 0	Day 14	Day 0	Day 14
		100 µg/mL			
T_{max}	hr	48	42	54	36
C_{max}	ng/mL	128	117	105	177
AUC $_{(0-t)}$	ng hr/mL	12100	11500	10300	17400
AUC $_{(0-\infty)}$	ng hr/mL	13000	12900	11300	18200
$t_{½,z}$	hr	33.7	38.1	35.9	41.4
		500 µg/mL			
T_{max}	hr	42	36	42	48
C_{max}	ng/mL	108	126	144	129
AUC $_{(0-t)}$	ng hr/mL	10700	12600	13400	12000
AUC $_{(0-\infty)}$	ng hr/mL	11600	13800	14600	13000
$t_{½,z}$	hr	35.0	38.9	35.6	33.9

[a] Numbers rounded to three significant figures

[b] Mean values estimated by non-compartmental analysis (n=3-4)



12. PK of NESP, [redacted]-NESP, & NESP[redacted] Following a Single IV Bolus to Male [redacted] Rats

Species: [redacted] rats (12 males/grp)

Dose Levels: 30 µg/kg

Route/Duration: single IV bolus

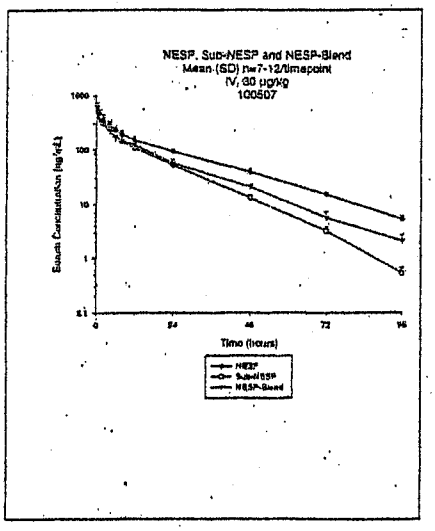
The material used were all glycosylated mixtures with different amounts of [redacted] NESP > NESP[redacted]-NESP [the > the amount of [redacted] the > the MW]

Methods: Rats received either NESP, [redacted]-NESP, or NESP-[redacted]. Blood samples were obtained up to 96 hrs postdose. The samples were assayed via [redacted] with a limit of quantitation of 0.180-0.25 ng/mL for NESP, 0.125-0.200 ng/mL for [redacted]-NESP, & 0.125-0.250 ng/mL for NESP-[redacted]

Findings: NESP clearance was inversely related to [redacted] content & the values were statistically significantly different: 4.27 ±0.263 mL/hr/kg (NESP), 6.78 ±0.589 mL/hr/kg [redacted]-NESP), 6.15 ±0.538 mL/hr/kg (NESP-[redacted].

Compound	NESP	Sub-NESP	NESP-Blend
Group	1	2	3
Parameter Estimates[a]:			
C_0 (ng/mL)	769	729	765
V_d (mL/kg)	41.2	42.5	42.0
$AUC_{(0-\infty)}$ (ng hr/mL)	7060	4460	4920
$t_{1/2,z}$ (hr)	17.5	11.0	14.4
CL (mL/hr/kg)	4.27	6.78	6.15
V_{ss} (mL/kg)	97.6	95.9	110

[a] averaged using non-compartmental analysis of individual animals, n=12 (n=11 for Group 3).



Preclinical Toxicology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. A Single Dose Toxicity Study of NM321 Administered IV to Rats; study #960006; performed at Amgen (non-GLP); lot [redacted] 11/96; part III of V, vol 2

2. Acute IV Toxicity Study with NM321 in Rats; study #970014; performed at [redacted] (per GLP); lot [redacted] 12/97; part III of V, vol 2

3. A Single Dose Range-Finding IV Toxicity Study of NM321 in Dogs; study #960011; performed at [redacted] (non-GLP); lot [redacted] 5/97; part III of V, vol 2

4. Acute IV Toxicity Study with NM321 in Dogs; study #970015; performed at [redacted] (per GLP); lot [redacted] 12/97; part III of V, vol 3

5. A 14-Day Toxicity Study of NM321 Administered IV & SC to Rats; study #960015; performed at Amgen (non-GLP); lot [redacted] 4/97; part III of V, vol 3

6. A 4-Week Toxicity Study of NM321 in the Rat via IV & SC Administration with a 2-Week Interim Sacrifice & a 4-Week Recovery Period; study #960026; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 4

7. 4-Week IV Toxicity Study of NM321 in Dogs with a 4-Week Recovery Period; study #960023; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 9

8. 4-Week SC Toxicity Study of NM321 in Dogs; study #960024; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 12

9. A 3-Month Toxicity Study of NM321 Administered via IV Injection in the Rat; study #960062; performed at [redacted] (per GLP); lot [redacted] 12/97; part III of V, vol 14

10. A 3-Month Toxicity Study of NM321 Administered via IV Injection in the Dog; study #960061; performed at [redacted] (per GLP); lot [redacted] 10/97; part III of V, vol 17

11. A 6-Month Toxicity Study of NM321 in the Rat via IV Administration with a 3-Month Interim Sacrifice & a 4-Week Recovery Period; study #960027; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 19

12. 26-Week IV Toxicity Study of NM321 in Dogs with a 7-Week Recovery Period; study #960025; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 25

13. Acute IV/IA/Perivenous/SC Tolerance Study with NESP in Rabbits; study #100488; performed at [redacted] (per GLP); lot [redacted] (HSA-free); 7/99; part III of V, vol 48

14. Assessment of the Hemolytic Property of NESP with Human Blood: Direct Contact Test; study #100489; performed at [redacted] (per GLP); lot [redacted] (HSA-free); 8/99; part III of V, vol 48

15. Determination of In Vitro Tissue Binding of EPO to Human Bone Marrow, Liver, & Pancreas; study #970143; performed at PAI (non-GLP); lot #0516B6A (EPO), lot [redacted] (NESP); 5/98; part III of V, vol 48

16. Limited Tissue In Vitro Binding Study of NESP & EPO to Human Tissues; study #100160; performed at [redacted] (non-GLP); lot [redacted] (EPO), lot [redacted] (NESP); 8/98; part III of V, vol 48

17. A Single Dose IV Toxicity Study with NESP & NESP-EL in Rats; study #100382; performed at [redacted] (per GLP); lot [redacted] (NESP), [redacted] (NESP [redacted]; 9/99; part III of V, vol 49

18. NESP: Consideration of Need for Carcinogenicity Testing; part III of V, vol 39

Acute Toxicity Studies

1. A Single Dose Toxicity Study of NM321 Administered IV to Rats
Species: [redacted] rats (5 females/grp)
Dose Levels: 0.135, 1.35, 6.7, 13.5 µg/kg [594 U of activity per µg peptide - doses of 80, 800, 4000, 8000 U/kg]
Route/Duration: IV/single injection + kill on day 15
Note that analysis revealed that actual doses were ~215, 515, 2700, 4900 U/kg.

Methods: Clinical signs, BWs, clinical pathology (baseline & days 7, 14), and gross evaluation

Findings: No effects on clin signs, BWs, & gross evaluation

↑HCT (~4-30%), retics (~3-15%), RBCs, HGB, MCV - Rx grps (dose-related); ↓PLTs - day 7 - all grps

↓neutrophils - ≥6.7 µg/kg - days 7, 14
No NOEL was noted.

2. Acute IV Toxicity Study with NM321 in Rats
Species: [redacted] rats (5/sex/grp)
Dose Levels: 0, 5, 30, 200 µg/kg
Route/Duration: IV/single injection + kill on day 16

Methods: Clinical signs, BWs, clinical pathology (days 8, 16), gross evaluation, & histopathology (femur/BM, sternum/BM, liver, spleen, & any lesions from controls & 200 µg/kg only)

Findings: No effects on clin signs, BWs

Day 8

↑(mild/moderate) RBCs, HGB, HCT - Rx grps - dose-related
↑(moderate/marked) retics - ≥30 µg/kg - dose-related
↑(slight) MCV, MCHC, MCH - Rx grps
↑PLTs - 200 µg/kg

Day 16

↑RBCs, HGB, HCT - ≥30 µg/kg
↓retics - ≥30 µg/kg
↓PLTs - 200 µg/kg

↓splenic erythropoiesis - 200 µg/kg

No NOEL was noted. Findings may have reflected nonspecific stimulation of thrombopoiesis, followed by a compensatory reduction of PLT production.

3. A Single Dose Range-Finding IV Toxicity Study of NM321 in Dogs
Species: [redacted] dogs (2/sex/grp)
Dose Levels: 0, 17, 34, 67 µg/kg
Route/Duration: IV/single injection + kill on day 15

Methods: Clinical signs, BWs, appetite, clinical pathology (baseline & days 4, 7, 10, 14), PK profile, and gross evaluation

Findings: No effects on clin signs, BWs, appetite

↑HGB, HCT, RBCs, retics, ↓serum Fe - Rx grps; seen with ≥34 µg/kg through day 14

↑WBCs, neutrophils & ↓BUN - Rx grps - day 4
↓BUN - 67 µg/kg - day 7
↓MCV - 67 µg/kg - day 14

↓glucose; ↑globulin, CK - ≥34 µg/kg - day 7/10
↑potassium - ≥34 µg/kg - days 7, 10, 14

PK - biphasic profile; mean terminal elimination half-life = 35.5 hrs

Preliminary serum EC_{50} = 75 ng/mL

4. Acute IV Toxicity Study with NM321 in Dogs
Species: [redacted] dogs (3 males/grp)
Dose Levels: 0, 15, 50, 150 µg/kg
Route/Duration: IV/single injection + kill on day 17

Methods: Clinical signs, BWs, appetite, clinical pathology (baseline & days 4, 7, 10, 14), gross evaluation, & histopathology (femur/BM, sternum/BM, liver, rib/BM, spleen from controls & 150 µg/kg only)

Findings: No effects on clin signs, BWs, appetite

↑RBCs, HGB, HCT - Rx grps - dose-related - peak at day 14 (last timepoint) - due to ↑production of smaller erythrocytes (↓ MCV) w/ ↓HGB (↓ MCHC) & a functional Fe deficiency [inability to mobilize/use adequate amounts of Fe needed for the rapid RBC production]

↓MCV, MCHC, MCH - Rx grps
↑retics - ≤50 µg/kg - days 4, 7 (peak)
↑retics - 150 µg/kg - day 10
↑retics - ≥50 µg/kg - day 14
↑WBCs, neutrophils - Rx grps - day 4

Splenic extramedullary hematopoiesis - 150 µg/kg

No NOEL was noted.

Multidose Toxicity Studies

5. A 14-Day Toxicity Study of NM321 Administered IV & SC to Rats
Species: [redacted] rats (5/sex/grp)
Dose Levels: 0, 0.135, 1.35, 6.7, 13.5 µg/kg/day (IV); 0, 1.35, 6.7, 13.5 µg/kg/day (SC)
Route/Duration: IV/SC injection/14 days
Note that analysis revealed that actual doses were ~11-27% lower than the targeted [listed above] dose levels. Actual dose levels = 0.12, 1.02, 5.24, 9.80 µg/kg/day (wk 1); 0.10, 1.06, 4.98, 9.89 µg/kg/day (wk 2)

Methods: Clinical signs, BWs, food consumption, clinical pathology (baseline & days 7, 14), PK profile, anti-NM321 Abs, and gross evaluation

Findings: One 1.35 µg/kg, IV male died on day 15. Day 14 observations included tremors, chromorhinorrhea; day 15 data included hypoactivity, loss of righting reflex, dilated pupils, cold to touch. The animal had dilated renal pelves, enlarged bladder

↓BWs (4-10%) - ≥1.35 µg/kg males, IV - days 6/13

PK - Accumulation ratios = 1.5/2.1 for IV/SC

IV - ↑HCT [~26-56%; dose-related], retics, RBCs, HGB, MCV - Rx grps (dose-related) - days 7, 14; with ↓MCV, MCH at day 14
↑PLTs - day 7; ↓PLTs - day 14 - all grps

↑ RDW% (RBC distribution width) - 4-14% - day 7 - Rx males, with slight ↓by day 14

↑WBCs - ≥6.7 µg/kg - day 7; with ↓ on day 14 (females)

SC - ↑ HCT [~18-32%; dose-related], retics, HGB, RBCs, MCV, WBCs, neutrophils - Rx grps (dose-related) - days 7, 14; with ↓ MCV, MCH, PLTs at day 14

↑RDW% - day 7 - Rx grps

Enlarged spleen - ≥1.35 µg/kg - IV/SC

6. A 4-Week Toxicity Study of NM321 in the Rat via IV & SC Administration with a 2-Week Interim Sacrifice & a 4-Week Recovery Period

Species: [redacted] rats (15/sex/grp-SC; 15-20/sex/grp-IV; 8/sex/grp-PK)

Dose Levels: SC - 0, 1, 100 µg/kg/dose; IV - 1, 30, 100 µg/kg/dose

Route/Duration: IV/SC injection/tiw for 4 wks + kills at wk 2,4 & recovery (wk 9 - IV control, 1, 100 µg/kg only)

Methods: Clinical signs, BWs, food consumption, ophthalmology, clinical pathology (day 9, wks 3, 4, 8), PK profile, anti-NM321 Abs, and gross & histopathologic evaluation

Findings:

Deaths - 2 rats (1 µg/kg, SC) - day 9; one rat (100 µg/kg, SC) - day 15; 2 rats (100 µg/kg, IV) - days 26 & 30 (due to pulmonary edema)

Red/thickened ears - 100 µg/kg, SC & IV [due to the ↑level of RBCs] - recovery

↓ BW gains, food consumption - ≥30 µg/kg males, IV; 100 µg/kg males, SC - recovery

Abs - (via an RIA) - wk 5 seropositive - 0/20 (IV control), 0/20 (SC control), 0/20 (1 µg/kg, IV), 2/18 (1 µg/kg, SC), 1/20 (30 µg/kg, IV), 2/19 (100 µg/kg, IV), 0/10 (100 µg/kg, SC)

PK - (via [redacted] - terminal half-life for IV = 14 hrs absorption half-life for SC - ~11 hrs; C_{max} = 3.17/320 ng/mL for 1/100 µg/kg; T_{max} = 12 hrs; bioavailability = 34.4%

Accumulation ratios were 1.0/1.1 for IV/SC routes

Clinical Pathology

↑HCT (18-32%), HGB (14-26%), RBCs (13-42%), retics (42-84%);
↓MCH, MCHC - Rx grps - day 9, wks 2, 4 [reflective of Fe depletion, resulting in undersized, ↓ HGB-containing RBCs]
↑PLTs - ≥30 µg/kg, IV/SC - day 9, wks 2, 4 - recovery

nRBCs, HJ bodies, Pappenheimer bodies -≥30 µg/kg,IV; 100 µg/kg,SC -trend toward recovery

↑PT (18%), APTT (131%) - 100 µg/kg, IV/SC - wks 2, 4 - recovery
↑WBCs - 100 µg/kg, IV/SC - day 9
↑neutrophils - all IV males, 100 µg/kg females, IV - day 9
↑lymphocytes - 100 µg/kg females, IV; 100 µg/kg, SC - day 9

↓serum Fe - Rx grps - wks 2, 4; followed by ↑ at recovery (IV)
↑Fe-binding capacity (mainly unsaturated) - Rx grps - wks 2, 4; followed by ↓ at recovery (IV)
[due to incorporation of Fe into HGB during the production of RBCs, followed by a rebound effect during recovery]

↑AST (50-100%) - ≥30 µg/kg, IV/SC - wks 2, 4 - recovery
↑BUN - ≥30 µg/kg, IV/SC - wks 2, 4 - recovery
↓glucose - Rx males, IV - wks 2, 4 - recovery

↓phosphorus (100 µg/kg males, SC - wk 2), cholesterol (≥30 µg/kg, IV/SC - wks 2, 4), albumin & total protein (100 µg/kg, IV/SC - wks 2, 4) - recovery
↓urine electrolytes - ≥30 µg/kg, IV/SC - wks 2, 4 - recovery

Gross

↑ spleen, brain weights - ≥30 µg/kg, IV/SC - wks 2, 4 - trend toward recovery

Spleen - enlarged - ≥30 µg/kg, IV/SC - wks 2, 4 - remained enlarged at recovery for 100 µg/kg, IV
Ears, lungs, liver - discolored - Rx grps - wks 2, 4 - recovery [due to vascular congestion]

Histo

BM - ↑ erythroid cells, with some BM cells noted in veins in the soft tissue adjacent to the sternum/femur - Rx grps - wks 2,4 - trend toward recovery

Spleen - extramedullary hematopoiesis (erythroid) - Rx grps - wks 2,4 - trend toward recovery

Liver - extramedullary hematopoiesis (erythroid) - Rx grps - wks 2,4 - recovery

Injection Site - subacute/chronic inflammation - all grps - recovery

Congestion of liver, brain, trachea, heart, LNs, lungs, spleen, kidneys, GI tract, gonads, adrenals, etc... - 100 µg/kg, IV/SC - wks 2,4 [due to ↑ erythroid cells]

The NOAEL was ≥100 g/kg/dose (20,000 U/kg/dose) for both routes. A NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

7. 4-Week IV Toxicity Study of NM321 in Dogs with a 4-Week Recovery Period

Species: [redacted] dogs (7-10/sex/grp)

Dose Levels: 0, 1, 20, 50 µg/kg/dose

Route/Duration: IV injection/tiw for 4 wks + kills at 2 & 4 wks (all grps) and 8 wks (control, 1, 50 µg/kg only)
Dogs were given 6 days of oral (50 mg/dog/day) Fe supplementation starting 8 days prior to start of dosing.

Methods: Clinical signs, BWs, food consumption, ophthalmology, clinical pathology (baseline & twice weekly), PK profile, anti-NM321 Abs, and gross & histopathologic evaluation

Findings:

Deaths - one 50 µg/kg male - day 33
Up to 2 wks prior to death - anorexia, ↓ BW (2 kg), dehydration, progressive deterioration, ↑ WBCs (neutrophils), ↓ PLTs, electrolyte imbalance. The dog had hemorrhage of the SC tissue in the ventral cervical area into the thoracic cavity - likely due to jugular venipuncture

↓ BWs, food consumption - ≥20 µg/kg - recovery

Abs - wk 2 - 1/6 (1 µg/kg), 1/6 (20 µg/kg) seropositive
wk 4 - 2/8 (1 µg/kg), 3/8 (20 µg/kg), 4/8 (50 µg/kg) seropositive
wk 8 - 2/5 (50 µg/kg) seropositive

PK -

Mean Non-Compartmental Pharmacokinetic Parameters Estimated for NM321 in Beagle Dogs During 4 Weeks of IV Dosing.

Dose µg/kg	Study Day	Dose Number	C_{max} ng/mL	$AUC_{(0-t)}$ ng*hr/mL	$t_{1/2,z}$ hr
1	1	1	13.76	178.9	18.32
	22	10	14.96	168.23	17.04
20	1	1	293.99	4873.6	30.09
	22	10	360.74	6194.6	25.44
50	1	1	642.33	11767.6	34.62
	22	10	914.54	16878	39.35

Accumulation ratios = 1.08/1.17/1.32 for 1/20/50 µg/kg
Disposition was triphasic

Dose day #1 - ↓ clearance with ↑ dose - due to saturation of Michaelis-Menten clearance process

Clinical Pathology

↑ HCT, HGB, RBCs, retics, nRBCs; ↓ MCV, MCH, MCHC - Rx grps - days 4, 9, 12, 16, 18, 23, 26, 30; recovery except for RBCs, MCH, MCV, MCHC [due to long life of the RBCs & the lack of HGB for production of new RBCs]

↑ WBCs, neutrophils, lymphocytes - ≥20 µg/kg - Rx interval - recovery
↓ PLTs - due to very large PLT size, preventing accurate automated determination

↓serum Fe - Rx grps; ferritin levels not changed - thus total body Fe was not depleted - day 4 thru Rx - recovery [insufficient Fe availability resulted in microcytic, hypochromic RBCs, thus the HGB & HCT increases were not huge]

↑protein, globulin, triglycerides; ↓ glucose BUN, ALT, A/G ratio - ≥20 µg/kg - Rx interval - recovery

↑cholesterol, CK - ≥20 µg/kg males; ↓total Fe binding capacity - 50 µg/kg females - Rx interval - recovery

↑potassium - Rx grps - Rx interval - recovery [likely due to leakage of intracellular K+ from ↑ RBCs during sample collection] - recovery

Gross

↑spleen weights; ↓ thymus weights - Rx grps (dose-related) - wks 2, 4 - recovery

Spleen - enlarged - ≥20 µg/kg - wks 2, 4 - recovery

Histo

Spleen - extramedullary hematopoiesis (erythroid, megakaryocytic) - ≥20 µg/kg - wks 2,4 - recovery

BM - ↑cellularity (↓ visible fat cells in stroma) - Rx grps - wks 2, 4 - recovery
↑ immature hematopoietic cells (erythroid, myeloid) at wk 2; with erythroid predominant + megakaryocytes at wk 4 - ≥20 µg/kg - recovery

Dilated vascular sinuses (congestion) - Rx grps - wks 2, 4

Myelofibrosis - ≥20 µg/kg - wks 2, 4; progressive with time - in some cases replacing extensive areas of BM, with occasional spicules of osteoid - seen in 2/4 (50 µg/kg) at recovery

Liver - hematopoietic cells & megakaryocytes in sinusoids - ≥20 µg/kg - wks 2, 4 - recovery

LNs - hematopoietic cells & megakaryocytes in sinuses - ≥20 µg/kg - wks 2, 4 - recovery

Kidneys - infarct - one 20 µg/kg female - wk 4; one 1 µg/kg female at recovery

Injection Site - hemorrhage, inflammation - all grps - recovery

The NOAEL was 1 µg/kg/dose, tiw (200 U/kg/dose). A NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

8. 4-Week SC Toxicity Study of NM321 in Dogs
Species: [redacted] dogs (7/sex/grp)
Dose Levels: 0, 1, 50 µg/kg/dose
Route/Duration: IV injection/tiw for 4 wks + kills at 2 & 4 wks
Dogs were given 7 days of oral (50 mg/dog/day) Fe supplementation starting 9 days prior to start of dosing.

Methods: Clinical signs, BWs, food consumption, ophthalmology, clinical pathology (baseline & days 4, 9, 12, 16, 18, 23, 26), PK profile, anti-NM321 Abs, and gross & histopathologic evaluation

Findings:
Abs - No Ab formation

PK -

Toxicokinetics of NM321 in [redacted] Dogs During 4 Weeks of SC Dosing (1 and 50 µg/kg TIW).

Dose µg/kg	Study Day	Dose Number	T_{max} hours	C_{max} ng/mL	AUC* ng*hr/mL
1	1	1	25.71	2.00	409.6
	24	12	13.50	5.71	229.3
50	1	1	27.43	162.3	713191
	24	12	19.50	610.2	25334

*$AUC_{0-\infty}$ for Day 1, $AUC_{\tau,ss}$ for Day 24

Accumulation ratios = 2.9/3.8 for 1/50 µg/kg
Profile was 2-compartment disposition, with first-order absorption

Dose day #1 - ↓clearance with ↑dose - due to saturation of Michaelis-Menten clearance process
Half-life = ~65 hrs

Clinical Pathology
↑HCT, HGB, RBCs, retics, nRBCs; ↓MCV, MCH, MCHC - Rx grps (dose-related) - Rx interval

↑WBCs, neutrophils, lymphocytes - 50 µg/kg - Rx interval

↓serum Fe - Rx grps; ferritin levels not changed - thus total body Fe was not depleted - day 4 thru Rx interval [insufficient Fe availability resulted in microcytic, hypochromic RBCs, thus the HGB & HCT increases were not huge]

↑protein, globulin, triglycerides; ↓ glucose, ALT - 50 µg/kg - Rx interval

↑cholesterol, CK - ≥20 µg/kg males; 9 total Fe binding capacity - 50 µg/kg females - Rx interval - recovery

↑potassium - Rx grps - Rx interval - recovery [likely due to leakage of intracellular K+ from ↑RBCs during sample collection] - recovery

Gross

↑spleen weights - 50 µg/kg - wks 2, 4

Spleen - enlarged - Rx grps (dose-related incidence) - wks 2, 4

Histo

Spleen - extramedullary hematopoiesis (erythroid, megakaryocytic) - Rx grps (dose-related) - wks 2, 4 (↓severity)

BM - ↑ cellularity (↓ visible fat cells in stroma) - Rx grps - wks 2, 4

↑ immature hematopoietic cells (erythroid, myeloid) at wks 2, 4 (↓ severity) - Rx grps; with erythroid predominant + megakaryocytes at wks 2, 4 - 50 µg/kg

Dilated vascular sinuses (congestion) - Rx grps - wks 2, 4

Myelofibrosis - ↑ perivascular collagen distribution w/ extension into the marrow; some with focal osteoid production - 50 µg/kg - progressive w/ time - 4/6 (wk 2) & 6/8 (wk 4); ostosis - 2/6 (wk 2) & 5/8 (wk 4)
Note that many of the marrows were normocellular

Liver - extramedullary hematopoiesis (erythroid) - Rx grps (dose-related) - wks 2, 4 (↓ severity)

Gallbladder, Mandibular LNs - extramedullary hematopoiesis (erythroid) - 50 µg/kg - wks 2, 4

Injection Site - hemorrhage, inflammation, fibrosis, fibrinoid vascular necrosis - all grps

The NOAEL was 1 µg/kg/dose, tiw (200 U/kg/dose). A NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

9. A 3-Month Toxicity Study of NM321 Administered via IV Injection in the Rat

Species: [redacted] rats (10/sex/grp - tox; 8/sex/grp-PK)

Dose Levels: 0, 1.5, 7.5, 30 µg/kg/dose, qiw; 0.5 µg/kg/dose, tiw

Route/Duration: IV injection/tiw & qiw for 13 wks + kills at wk 13

Methods: Clinical signs, BWs, food consumption, ophthalmology, clinical pathology (day 9, wks 5, 13), PK profile (days 1 & 85), anti-NM321 Abs, and gross & histopathologic evaluation

Findings: Deaths - Tox - 1/20 (placebo); 3/20 (7.5 µg/kg, qiw); 7/20 (30 µg/kg, qiw)
PK - 2/16 (1.5 µg/kg, qiw); 1/16 (7.5 µg/kg, qiw); 3/16 (30 µg/kg, qiw)

Red ears - Rx grps; time of onset was dose-related - noted after 5 wks (30 µg/kg), 7 wks (7.5 µg/kg), 8-10 wks (1.5 µg/kg) Incidence in qiw rats was dose-related; with highest incidence noted in the tiw rats

↓ BWs - Rx males - 7%, 9%. 17% for 1.5, 7.5, 30 µg/kg (qiw) & 14% for 0.5 µg/kg (tiw) at wk 13

Retinal & choroidal congestion - Rx grps - wk 13
Unilateral intravitreal hemorrhage - one 7.5 µg/kg &

Abs - 3 months - 2/20 (1.5 µg/kg), 2/17 (7.5 µg/kg), 4/13 (30 µg/kg), 3/18 (0.5 µg/kg, tiw) - seropositive

PK -

Table 2: Primary Non-compartmental Parameters for Each Group on Day 1 and Day 85

Parameter	Units	Group 2 1.5 µg/kg IW	Group 3 7.5 µg/kg IW	Group 4 30 µg/kg IW	Group 5 0.5 µg/kg TIW
Day 1					
C_{max}	ng/mL	44.2	149	652	11.3
$AUC_{0-\infty}$	ng hr/mL	338	1800	7580	104
$t_{1/2}$	hr	16.3	18.7	17.0	14.0
V_z	mL/kg	94.4	95.4	84.9	90.4
CL	mL/hr/kg	4.44	4.15	3.96	4.81
Day 85					
C_{max}	ng/mL	53.8	238	784	14.8
$AUC_{0-\infty}$	ng hr/mL	492	2190	8890	139
$t_{1/2}$	hr	13.0	15.4	19.4	13.2
V_z	mL/kg	49.0	61.1	73.1	51.5
CL	mL/hr/kg	3.05	3.42	3.37	3.59
AR*	%	1.46	1.21	1.17	1.34

*AR - Accumulation ratio, calculated from:

$$AR_{0-\infty} = \frac{AUC_{(0-\infty),Day\,85}}{AUC_{(0-\infty),Day\,1}}$$

Terminal half-life = ~15 hrs
Clearance = ~4 mL/hr/kg for all Rx grps
Accumulation ratio = 1.46/1.21/1.17 for 1.5/7.5/30 µg/kg, qiw and 1.34 for 0.5 µg/kg, tiw

Clinical Pathology - ↑ HCT, HGB, RBCs, retics, MCV; ↓MCHC - Rx grps (dose-related) - day 9, wks 5, 13 - changes more pronounced w/ 0.5 µg/kg tiw than 1.5 µg/kg qiw

↓ PLTs; ↑ PT, APTT - Rx grps - wks 5, 13
↓ serum Fe; ↑ unsaturated iron-binding capacity, total iron binding capacity - Rx grps - wk 5 [reflective of Fe incorporation into HGB in response to RBC production]

↑ AST, BUN, bilirubin, phosphorus, potassium; ↓ glucose, urinary electrolytes - Rx grps - wk 5/13
Red-tinged urine - latter part of study - two rats (30 µg/kg, qiw) & one rat (0.5 µg/kg, tiw)

↑ spleen weights - Rx grps
↑ heart, lungs, adrenals, liver weights - 30 µg/kg females (qiw), 0.5 µg/kg females (tiw)

Discoloration - skin, kidneys, liver, stomach, lung - Rx grps
Stomach thickening - ≥7.5 µg/kg (qiw); 0.5 µg/kg (tiw)
Enlarged spleen - ≥7.5 µg/kg (qiw); 0.5 µg/kg (tiw)

BM (sternum/femur) - hypercellularity (minimal/moderate) - due to ↑ erythropoiesis - the 0.5 µg/kg tiw grp showed an enhanced response compared to the 1.5 µg/kg qiw grp

Femoral BM necrosis/fibrosis - 1/20 females (30 µg/kg), 1/20 females (0.5 µg/kg, tiw)

Spleen - lymphoid atrophy, extramedullary hematopoiesis - ≥7.5 µg/kg, 0.5 µg/kg females (tiw)

Thymus - lymphoid atrophy (mild) - Rx grps - dose-related

Stomach (glandular) - ↑ erosion, hemorrhage, edema, congestion, inflammatory infiltrates - Rx grps - dose-related

Heart - ↑ incidence of focal myocardial degeneration - in left ventricle/interventricular septum (minimal/mild) - 1/20 (placebo), 2/20 (1.5 µg/kg), 5/20 (7.5 µg/kg), 11/20 (30 µg/kg), 2/20 (0.5 µg/kg, tiw)

Kidney - necrosis (unilateral, moderate/marked) - 1/10 females (30 µg/kg), 2/10 males (0.5 µg/kg, tiw)

Lung - ↑ incidence of hemorrhage - Rx grps [due to combination of euthanasia + polycythemia]

Congestion in many tissues [due to polycythemia]

The NOAEL was 1.5 µg/kg/wk [1.5 µg/kg/dose, qiw or 0.5 µg/kg/dose, tiw]. A NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

10. A 3-Month Toxicity Study of NM321 Administered via IV Injection in the Dog

Species: beagle dogs (3-4/sex/grp)

Dose Levels: 0, 1.5, 7.5, 30 µg/kg/dose, qiw; 0.5 µg/kg/dose, tiw

Route/Duration: IV injection/qiw/tiw for 13 wks + kills at wk 14

Methods: Clinical signs, BWs, food consumption, ophthalmology, ECGs, clinical hematology (baseline, wks 2, 5, 14), clinical chemistry (baseline, wks 5, 14), PK profile (day 1, wks 5 & 13), anti-NM321 Abs, and gross & histopathologic evaluation

Findings:

Injection of scleral blood vessels - Rx grps (predominantly in the qiw grps) - month 3 [due to the elevated circulating RBCs]

Anaphylactic rxn [vocalization, prostration, ataxia, swollen skin, emesis, salivation, lacrimation, urination, nasal discharge] to vehicle (0.25% HSA) - control & Rx grps - beginning on day 22 - given epinephrine + antihistamine to control rxn Dosing continued & some animals (including controls) continued to display rxn until study terminated

↓ BWs - 30 µg/kg (qiw) females [12% lower than controls by 3 months]

↓ BWs - Rx males - sporadic

Abs - detected in 1/8 controls (wk 12); 1/6 dogs (1.5 µg/kg, wks 8,12); in 30 µg/kg males - wks 8, 12

PK -

Table 2: Summary Non-compartmental Parameters for Each Group on Days 1, 29 and 85

Group	Parameter	Units	Day 1		Day 29		Day 85	
			Mean	SD	Mean	SD	Mean	SD
2 (1.5 µg/kg)	Co	ng/mL	30.71	3.891	34.29	4.054	39.50	3.822
	$AUC_{0-\infty}$	ng hr/mL	603.3	53.30	564.6	96.36	676.5	67.79
	$nAUC_{0-\infty}$	ng hr/mL/(µg/kg)	402.2	35.53	376.4	64.24	451.0	45.19
	$MRT_{0-\infty}$	hr	31.25	5.622	25.92	3.130	26.62	2.677
	$t_{1/2,z}$	hr	22.91	4.254	18.71	2.245	19.30	2.131
	V_z	L/kg	0.0774	0.0093	0.0695	0.0048	0.0592	0.0045
	CL	L/hr/kg	0.0025	0.0002	0.0027	0.0005	0.0022	0.0003
	AR*	—	—	—	0.9419	0.1819	1.126	0.1278
3 (7.5 µg/kg)	Co	ng/mL	143.6	17.02	190.3	25.66	214.5	24.39
	$AUC_{0-\infty}$	ng hr/mL	3966	347.7	3160	390.1	3453	369.1
	$nAUC_{0-\infty}$	ng hr/mL/(µg/kg)	528.8	46.4	421.3	52.01	460.3	49.21
	$MRT_{0-\infty}$	hr	45.21	6.683	30.40	2.878	29.36	3.232
	$t_{1/2,z}$	hr	32.42	4.933	22.39	2.516	21.71	2.631
	V_z	L/kg	0.0855	0.0096	0.0726	0.0075	0.0640	0.0058
	CL	L/hr/kg	0.0019	0.0002	0.0024	0.0003	0.0022	0.0002
	AR*	—	—	—	0.6503	0.3218	0.8725	0.0852
4 (30 µg/kg)	Co	ng/mL	573.4	47.8	786.4	58.36	800.4	173.3
	$AUC_{0-\infty}$	ng hr/mL	18090	822.2	15390	1545	16010	1803
	$nAUC_{0-\infty}$	ng hr/mL/(µg/kg)	603.1	27.4	513.0	51.46	533.6	60.11
	$MRT_{0-\infty}$	hr	54.46	6.356	38.17	5.264	37.97	6.779
	$t_{1/2,z}$	hr	39.19	4.904	27.92	3.804	27.96	4.932
	V_z	L/kg	0.0904	0.0103	0.0743	0.0056	0.0715	0.0129
	CL	L/hr/kg	0.0017	0.0001	0.0020	0.0002	0.0019	0.0002
	AR*	—	—	—	0.7359	0.3072	0.8844	0.0885
5 (0.5 µg/kg)	Co	ng/mL	9.761	0.9853	11.67	1.859	13.07	1.779
	$AUC_{0-\infty}$	ng hr/mL	146.9	18.09	133.0	9.677	135.1	21.63
	$nAUC_{0-\infty}$	ng hr/mL/(µg/kg)	293.7	36.18	266.0	19.35	270.1	43.25
	$MRT_{0-\infty}$	hr	22.20	2.806	16.83	1.964	14.64	3.541
	$t_{1/2,z}$	hr	16.21	1.932	12.39	1.384	11.43	2.048
	V_z	L/kg	0.0760	0.0078	0.0634	0.0066	0.0535	0.0065
	CL	L/hr/kg	0.0035	0.0005	0.0038	0.0003	0.0038	0.0008
	AR*	—	—	—	0.9148	0.1031	0.9363	0.2017

*AR - Accumulation ratio, calculated from:

$$AR_{Day29} = \frac{AUC_{(0-\tau),Day29}}{AUC_{(0-\tau),Day1}} \text{ and } AR_{Day85} = \frac{AUC_{(0-\tau),Day85}}{AUC_{(0-\tau),Day1}}$$

Terminal half-life = [day 1] 16.2 hrs at 0.5 µg/kg; 39.2 hrs at 30 µg/kg
Clearance = [day 1] 3.5 mL/hr/kg at 0.5 µg/kg; 1.7 mL/kg/hr at 30 µg/kg
Accumulation ratio= 1.1 for 1.5 µg/kg, qiw and 0.9 for 0.5 µg/kg, tiw

Clinical Pathology - ↑HCT, HGB, RBCs, retics; ↓MCHC, MCH, MCV - Rx grps (dose-related) - wks 2/5, 14
Changes more pronounced w/ 0.5 µg/kg tiw than 1.5 µg/kg qiw

↑PLTs, PT, APTT - Rx grps (dose-related) - wks 5, 14
↓serum Fe; ↑unsaturated iron-binding capacity - Rx grps - wks 5, 14

↑AST, ALT (one 30 µg/kg male) - wk 5
↑ phosphorus, potassium - Rx grps - wks 5, 14
↓glucose - ≥7.5 µg/kg - wks 5, 14
↓albumin - 30 µg/kg - wk 14

↑kidney weights - 30 µg/kg
Discoloration - eyes, GI tract, kidneys, injection site

Congestion in many tissues [due to polycythemia]

BM - [sternum & femur] mild hypercellularity (due to ↑ erythropoiesis) - 30 µg/kg - more pronounced w/ 0.5 µg/kg tiw than 1.5 µg/kg qiw

Myelofibrosis (mild/moderate); deposition of collagenous matrix with some mineralization - 30 µg/kg

Osseous hyperplasia - 30 µg/kg

Spleen, Liver - extramedullary hematopoiesis - 30 µg/kg

Kidneys - ↑ glomerular mesangial matrix (eosinophilia of glomerular tuft) - ≥7.5 µg/kg & 0.5 µg/kg, tiw

↑tubular degeneration (minimal/mild) - 30 µg/kg & 0.5 µg/kg, tiw
Fibrin thrombi in glomerular capillaries - 30 µg/kg

The NOAEL was 1.5 µg/kg/dose, qiw. A NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

11. A 6-Month Toxicity Study of NM321 in the Rat via IV Administration with a 3-Month Interim Sacrifice & a 4-Week Recovery Period

Species: [redacted] rats (15-20/sex/grp - tox; 8/sex/grp-PK)
Dose Levels: 0, 1, 5, 20 µg/kg/dose
Route/Duration: IV injection/tiw for 26 wks + kills at 3 months (control, 1, 5 µg/kg only), 6 months (all grps), & 7 months (all grps, recovery)

On day 40, doses were lowered to 0, 0.5, 2.5, & 10 µg/kg/dose due to mortality at 20 µg/kg. On day 47, the dosing regimen was changed from tiw to qiw at doses of 0, 1.5, 7.5, & 30 µg/kg/dose - the weekly equivalent of tiw dosing.

In this review, the original grps will be designated as I, II, III, IV

Methods: Clinical signs, BWs, food consumption, ophthalmology, clinical pathology (day 9, [approximate] wks 4, 7, 12, 15, 26, 30), PK profile, anti-NM321 Abs, and gross & histopathologic evaluation
Note that satellite rats were dosed for the PK samples

Findings: Tox study
Deaths - 7/15 males & 3/15 females (grp III) - days 68-183
12/20 males & 17/20 females (grp IV) - days 32-177; & 3 males died of accidental causes [grp IV, specifics not given]
There were also deaths in grps III & IV in the PK study

Findings due to the high viscosity of the blood involved the heart (thrombosis, inflammation, fibrosis, intimal hyperplasia of the atrio-ventricular/aortic valves); hypertrophy/hyperplasia (Kupffer cells)
Additional findings are listed under histo

Red/thickened ears - Rx grps - by day 25 (grps III, IV); by day 39 (grp II) - trend toward recovery
Red/thickened paws also noted - recovery
White incisors - grps III, IV - by day 39, peaking at days 46-53

↓ BW gains (15%) - grp IV males

Abs - (via an [redacted] month 6 - 2/~~10~~ 20 MMS 10/2/07 (grp II), 1/~~3~~ 5 (grp III) were seropositive

PK - AUC determinations indicated dose-linearity
Accumulation ratios ranged between 1.58-2.15 for days 94 & 187

↑HCT (19-43%), HGB (15-40%), RBCs (16-43%), retics (26-100%) - Rx grps (dose-related trend) - day 9 & months 1, 3, 6 - recovery

↓MCHC, ↑ MCV - Rx grps - day 9 & months 1, 3, 6

↓PLTs - Rx grps - months 1, 3, 6 - trend toward recovery

nRBCs, HJ bodies, Pappenheimer bodies - grps III, IV - day 9/ months 1, 3 - trend toward recovery

↑PT, APTT - Rx grps - months 1, 3, 6 - recovery
↑WBCs, neutrophils, lymphocytes - grp IV - day 9 & month 3 and grps III, IV - month 6 - trend toward recovery

↓serum Fe - Rx grps (dose-related) - months 1, 3, 6 & wk 15; followed by ↑at recovery
↑Fe-binding capacity (unsaturated & total) - Rx grps - month 1; variable at other intervals - followed by 9 trend at recovery

↑AST - Rx grps - months 1, 3, 6 & wks 7, 15 - recovery
↑AlkP - grps III, IV - wk 7
↑BUN, bilirubin - Rx grps - during Rx - recovery
↓glucose, cholesterol - Rx grps- months 1, 3, 6, & wks 7, 15 - recovery

↑phosphorus, potassium - grps III, IV - months 1, 3, 6, & wks 7, 15
↑beta globulin - grps III, IV - months 1, 3, 6

↑occult blood - Rx grps - months 1, 3, 6 [no ↑ RBCs/WBCs]
Red-tinged urine - grps III, IV - days 82-181 (sporadic)
↓urine electrolytes - Rx grps - months 3, 6 - recovery

↑ spleen weights - Rx grps - months 3, 6 - trend toward recovery
↑kidney, heart, liver weights - grps II, III - month 3/6- recovery
↑ovary weights - grps III, IV - months 3, 6 (due to vascular congestion)

Spleen - enlarged - Rx grps - months 3, 6 - recovery
Ears, lungs, liver, spleen - discolored - Rx grps - months 3, 6 - recovery [due to vascular congestion]
Kidneys - rough surfaces, discolored - grps III, IV - remained at recovery

Histo
BM - ↑erythroid cells, with some erythroid and/or BM cells noted in veins in the soft adipose tissue adjacent to the sternum/femur - Rx grps (dose-related) - months 3, 6 - trend toward recovery

Osseous hyperplasia (slight/moderate) - femur - Rx grps (dose-related) - month 6 - recovery

Spleen - extramedullary hematopoiesis (erythroid) - Rx grps - months 3, 6 (dose-related) - recovery for grps II, III

Mineral deposits in smooth muscle (capsule & trabeculae) - Rx grps - at recovery kill [thought to be due to splenic size, resulting in stress/damage to smooth muscle]

Heart - valvular thickening (slight/moderately severe), hemorrhages, thrombosis, inflammation, fibrosis, intimal hyperplasia (left atrio-ventricular/aortic valves) - grps III, IV - month 6 - [due to ↑ HCT increasing the viscosity of the blood] - recovery

Degenerated myocardial fibers, fibrosis (minimal/slight) - grp IV - month 6 - recovery

Liver - hypertrophy/hyperplasia (Kupffer cells - stimulated for removal of degenerated RBCs?), with foamy cytoplasm & cellular debris - grps III, IV - month 6 - recovery

Kidneys - tubular nephropathy (minimal/moderate) - grps III, IV (dose-related), one in grp II - months 3, 6 - recovery

Intracytoplasmic granules (eosinophilic/brown pigment) in tubular epithelium - grps III, IV (dose-related) - month 6 - trend toward recovery

Infarcts (slight/moderate), with congestion, hemorrhage, residual necrosis, dystrophic mineral deposits, regenerated basophilic tubules, replacement by fibrous connective tissue - grps III, IV - month 6 & in recovery (one in grp ~~II~~ I) MAS 10/6/03

GI Tract - hemorrhages (minimal/moderately severe) - grps III, IV (dose-related); stomach/cecum necrosis - grps III, IV - months 3, 6 - recovery
Erosions/ulcers in cecum - one in grp III -month 3 & two grp IV deaths

Brain - malacia (cerebral cortex) - one grp IV at month 6 & one grp IV death

Injection Site - hemorrhage, inflammation, fibrosis - all grps - recovery

Congestion & stasis of blood in many tissues (including above) - Rx grps - months 3, 6

The NOAEL was 1.5 µg/kg/dose (300 U/kg/dose), once weekly dosing. A NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

12. 26-Week IV Toxicity Study of NM321 in Dogs with a 7-Week Recovery Period

Species: [redacted] dogs (7-10/sex/grp)

Dose Levels: 0, 1, 5, 20 µg/kg/dose - supplemented with Fe & 20 µg/kg/dose - no Fe

Route/Duration: IV injection/tiw for 26 wks + kills at wks 5 (grp V), 14 (grps I-V), 27 (grp I-IV) & at recovery [wks 18 (grp V), 34 (grp I, II, IV)]

On day 57, doses were lowered to 0, 0.5, 2.5, 10, 10 µg/kg/dose due to mortality at 20 µg/kg. On day 71, the dosing regimen was changed from tiw to qiw at doses of 0, 1.5, 7.5, 30, 30 µg/kg/dose - the weekly equivalent of tiw dosing.
For all doses, dogs were given 7 days of oral (50 mg/dog/day) Fe supplementation starting 9 days prior to start of dosing.
Starting in wk 5, Fe was given daily.
There was also a separate high dose grp (grp V) that received no Fe. All surviving grp V dogs were killed at wk 18

In this review, the original grps will be designated as I, II, III, IV (all with Fe supplementation), and V (no Fe supplementation)

Methods: Clinical signs, BWs, food consumption, ophthalmology, ECGs, clinical pathology (baseline & days 4, 11, 16, 23, 37, 51, 65, 80, 93, 108, 121, 122, 136, 150, 164, 176, 192, 206, 221, 229), PK profile, anti-NM321 Abs, and gross & histopathologic evaluation

Findings: Deaths - grp III - one each at wk 9, 25
Grp IV - one each at wk 8, 10, 11 (2 dogs), 21, 24, 25 (2 dogs)
Grp V - one each at wk 8

The dogs displayed ataxia, seizures, limited use of limbs. Although not seen microscopically, blood clots/cerebral accidents were thought to cause the CNS toxicities

One grp IV female (moribund kill) was anorexic & had an area of infarction in the jejunum

One grp V female (found dead) had extensive hemorrhage in the heart, splenic enlargement, congestion of tissues, & a saddle thrombus in the iliac artery bifurcation

Although 4/5 dogs dying in wks 21-25 showed varying degrees of BM hypocellularity due to myelofibrosis, the red cell mass did not reflect diminished erythropoiesis.

Survivors - dark gums [hyperemia] & periodic lameness (hemorrhage noted in joint capsules) - Rx grps
Anaphylaxis [lip/ear/eyelid swelling, emesis, salivation, hypoactivity, facial edema, prostration, injected sclera] - all grps - ~wk 3 - noted postdose; severity & incidence diminished over time
[Formulation contains HSA]

↓ BWs, food consumption - grp IV, V - wks 1-14

↓ R/S amplitude (lead V_2), Q/R amplitude (lead V_{10}) - Rx grps (dose-related) - due to ↑ blood viscosity & ↑ cardiac load & from polycythemia, which interferes w/ coronary circulation, causing subendocardial underperfusion

Abs - dose-related incidence of seroreactivity - at least one dog per grp (including controls) - wk 4 thru dosing;
As many as 4/13 (grp III), 12/16 (grp IV), 7/13 (grp V) at wk 12

PK - not affected by Abs or by gender
Accumulation ratios ranged between 0.97-1.36 on day 85 & from 0.96-1.32 on day 183

Terminal half-life increased - 19.38 hrs (grp II), 36 hrs (grp IV) & clearance decreased - 3.9 mL/hr/kg (grp II), 2.2 mL/hr/kg (grp IV) - with increasing dose

Table 1:
Mean Non-Compartmental Parameters for NM321, estimated on Day 1, Day 85, Day 183 and Day 22 in Dogs Following Intravenous Administration (1, 5, 20 µg/kg TIW; 0.5, 2.5, 10 µg/kg TIW; 1.5, 7.5, 30 µg/kg IW) over 6 Months

Day 1					
Parameter	Units	Group 2	Group 3	Group 4	Group 5
C_0	ng/mL	14.68	73.85	333.6	307.5
$AUC_{0-\infty}$	ng hr/mL	214.0	1159	5579	5342
λ_z	hr^{-1}	0.0358	0.0267	0.0192	0.0194
$t_{1/2\lambda z}$	hr	19.38	25.96	36.02	35.70
AUC_{0-t}	ng hr/mL	258.1	1569	8903	8541
$nAUC_{0-t}$	ng hr/mL/(µg/kg)	258.1	313.9	445.1	427.1
V_z	L/kg	0.1083	0.1193	0.1167	0.1206
CL	L/hr/kg	0.0039	0.0032	0.0022	0.0023
MRT_{0-t}	hr	26.68	35.38	49.09	49.08
V_{ss}	L/kg	0.1034	0.1127	0.1103	0.1149

Day 85					
Parameter	Units	Group 2	Group 3	Group 4	Group 5
C_0	ng/mL	30.68	154.3	816.5	604.5
$AUC_{0-\infty}$	ng hr/mL	428.0	1942	9332	8514
λ_z	hr^{-1}	0.0337	0.0359	0.0253	0.0252
$t_{1/2\lambda z}$	hr	20.56	19.30	27.41	27.46
AUC_{0-t}	ng hr/mL	526.2	2329	12915	11757
$nAUC_{0-t}$	ng hr/mL/(µg/kg)	350.8	310.5	430.5	587.9
V_z	L/kg	0.0845	0.0897	0.0918	0.0674
CL	L/hr/kg	0.0029	0.0032	0.0023	0.0017
MRT_{0-t}	hr	28.06	25.97	36.65	36.82
V_{ss}	L/kg	0.08	0.0836	0.0851	0.0626

Table 1 (continued):

Day 183				
Parameter	Units	Group 2	Group 3	Group 4
C_0	ng/mL	30.19	159.1	730.7
AUC_{0-t}	ng hr/mL	432.6	2223	9773
λ_z	hr^{-1}	0.038	0.0343	0.028
$t_{1/2\lambda z}$	hr	18.25	20.21	24.75
AUC_{0-t}	ng hr/mL	510.9	2709	12862
$nAUC_{0-t}$	ng hr/mL/(µg/kg)	340.6	361.1	428.7
V_z	L/kg	0.0773	0.0807	0.0833
CL	L/hr/kg	0.0029	0.0028	0.0023
MRT_{0-t}	hr	25.15	27.29	33.04
V_{ss}	L/kg	0.0738	0.0756	0.0771

Day 22		
Parameter	Units	Group 5
C_0	ng/mL	397.9
$AUC_{0-\infty}$	ng hr/mL	7037
λ_z	hr^{-1}	0.0201
$t_{1/2\lambda z}$	hr	34.49
AUC_{0-t}	ng hr/mL	10980
$nAUC_{0-t}$	ng hr/mL/(µg/kg)	549.0
V_z	L/kg	0.0906
CL	L/hr/kg	0.0018
MRT_{0-t}	hr	46.98
V_{ss}	L/kg	0.0856

↑HCT, HGB, RBCs, retics, nRBCs, PT, APTT; ↓MCV, MCH, MCHC, urine electrolytes, urine specific gravity - Rx grps (dose-related)
↑WBCs, neutrophils, lymphocytes - grp III, IV, V

↓Fe - Rx grps

↑triglycerides, AST, CK, phosphorus, potassium; ↓glucose, chloride - Rx grps

↑globulin - grp IV, V
↑cholesterol - grps II, III, IV
↑AlkP - grp IV males

↓urine specific gravity, electrolytes - Rx grps
↑urine occult blood (Rx grps), urine protein (grp III, IV, V) - correlate with renal glomerular & tubular lesions
All clin path findings (except MCV, MCH, MCHC) resolved

Gross
Enlarged spleens, kidneys; darkened GI mucosa - grps III-V
↑spleen, kidney wts - grps III-V - interim & terminal kills - recovery with spleen

Histo
Deaths -
#Wk 14 - ↑hematopoiesis - spleen, BM
Myelofibrosis; ↑cancellous bone [hypocellular BM]
Kidney - ↑regenerative tubules (from polycythemia)

Wks 15-25 - ↑hematopoiesis - spleen, BM
Myelofibrosis; ↑cancellous bone [hypocellular BM]
Kidney - ↑regenerative tubules & glomerulopathy (from polycythemia & resulting tissue hypoxia)

Centrilobular degeneration/necrosis [due to tissue hypoxia] - liver of one grp IV female (wk 25)

Survivors -
Grp V - interim kill at wk 5; terminal kill at wk 14; recovery kill at wk 18
BM - myelofibrosis - wk 5; increasing at wk 14 (terminal kill) - present at recovery
↑bone in BM - wk 14 - present at recovery
[The ↑myelofibrosis at wk 14 indicated a diminished response of the BM to prolonged stimulation]

Spleen - ↑hematopoiesis - wks 5, 14 - trend toward recovery
Other - congestion in various tissues - wks 5, 14 - trend toward recovery

Grp III, IV - interim kill at wk 14; terminal kill at wk 27; recovery kill (grp IV only) at wk 34

↑hematopoiesis - spleen, BM - wks 14, 27 - recovery
Myelofibrosis - wks 14, 27 - present at recovery (grp IV)
↑bone in BM - wks 14, 27 - present at recovery (grp IV)

Kidney - ↑regenerative tubules & glomerulopathy - wks 14, 27 - grps III, IV - present at recovery
Liver - centrilobular necrosis/degeneration, fibrosis [due to tissue hypoxia] - wks 14, 27 - grps III, IV - recovery
Other - congestion in various tissues - wks 14, 27 - trend toward recovery

Grp II - interim kill at wk 14; terminal kill at wk 27; recovery kill at wk 34

Kidney - tubular changes - wk 14 - recovery
A NOAEL or a NOEL was not achieved, as exaggerated pharmacological responses were noted at all dose levels.

Other

13. Acute IV/IA/Perivenous/SC Tolerance Study with NESP in Rabbits

Species: [redacted] rabbits (6 males/grp)
Dose Levels: 100 µg/mL, 1 mL/kg (IV & IA); 478 µg/mL, 1 mL/site (SC); 478 µg/mL, 0.1 mL/rabbit (PV)
Route/Duration: IV/IA/PV/SC; single injection + kills on days 4 & 15

Methods: Clin signs (4 hrs postdose & daily), BWs, and gross & histopathologic evaluation (injection site only)

Findings: No findings were observed that were considered to be related to the test agent. Injection site reactions considered related to the actual dosing technique included hematomas (IV, IA, PV routes).

14. Assessment of the Hemolytic Property of NESP with Human Blood: Direct Contact Test

Anticoagulated human blood was mixed with 0.0185, 0.185, 1.85, or 18.5 µg/mL of NESP, followed by [redacted] minutes incubation at [redacted] and the [redacted] The hemolytic

index was 0.61% (vehicle), 0.040% (saline), -0.91% (0.0185 µg/mL), -1.29% (0.185 µg/mL), -1.15% (1.85 µg/mL), 0.34% (18.5 µg/mL). The agent NESP is non-hemolytic (scores of 2% are considered non-hemolytic).

15. Determination of In Vitro Tissue Binding of EPO to Human Bone Marrow, Liver, & Pancreas

In order to evaluate the potential ability of NESP to bind to cells expressing EpoR in human tissue. Bone marrow [expresses EpoR] and liver & pancreas [lack EpoR] were used. Various tissue fixation & signal detection methods were used in an attempt to optimize the assay. The optimized assay was able to reflect the binding of NESP to BM, but not to liver or pancreas. Refer to the study report for additional details.

16. Limited Tissue In Vitro Binding Study of NESP & EPO to Human Tissues

The ability of NESP (1 µg/mL) to bind to human tissues was evaluated. EPO (1 µg/mL) was used as the positive control. A total of 23 different tissues from two donors was used [refer to the study report for the list]. Both NESP & EPO showed binding to bone marrow only. Apparent nonspecific binding to mast cells in various tissues (intestine, lung, tonsil, spleen) was noted for NESP, EPO, NESP vehicle, & the assay control.

17. A Single Dose IV Toxicity Study with NESP & NESP-[redacted] in Rats

Species: [redacted] rats (10 males/grp = tox study; 9 males/grp = TK study)

Dose Levels: 0, 5, 30, 200, µg/kg/dose [NESP-EL]; 5, 200 µg/kg/dose [NESP]

Route/Duration: IV injection + kill on day 15

Methods: Clinical signs, BWs, food consumption, clinical pathology (hematology - days 7, 10, 15; chemistry - day 15), TK profile (3 males/timepoint), and gross & histopathologic evaluation (bone, BM, heart, kidney, liver, spleen, lesions)

Findings: No abnormalities - clinical signs, BWs, food consumption, gross or histopath

TK - serum concentration time profiles were comparable for both agents [CL = 3.37-3.64 mL/hr/kg for NESP-[redacted] & 2.99-3.35 mL/hr/kg for NESP]

Clinical Pathology

↑RBCs, HGB, HCT - day 7 (5 µg/kg, NESP-[redacted]; days 7, 10 (30 µg/kg, NESP-[redacted] & 5 µg/kg, NESP); days 7, 10, 15 (200 µg/kg, both agents - 15-20% for NESP & 9-11% for NESP-[redacted])
Note that NESP was more potent than NESP-[redacted] at equivalent dose levels

↓MCHC - days 7, 10, 15 - NESP grps; days 7,10 - NESP-[redacted] grps
↑MCV - day 7 - 200 µg/kg NESP; ≥30 µg/kg NESP-[redacted]
↓MCH - day 15 - 200 µg/kg NESP

↑polychromasia - day 7, 10 - NESP grps; day 7 -200 µg/kg NESP-[redacted]
↑WBCs, neutrophils, lymphocytes - day 7/15 - 200 µg/kg NESP
↑PLTs - day 7 - 200 µg/kg - both agents; ↓PLTs - day 15 - 200 µg/kg NESP

↑M/E ratio - day 15 - ≥5 µg/kg NESP; ≥30 µg/kg NESP-[redacted]
↑retics - day 7/10 - 200 µg/kg - both agents
↑total lymphoid cells - ≥5 µg/kg NESP; ≥30 µg/kg NESP-[redacted]
↓BM total granulocytic (myeloid) cells - 200 µg/kg NESP

[Possibly due to a negative feedback response, in which the higher number of circulating RBCs caused decreased numbers of RBCs in the BM & decreased levels of circulating retics]

Gross

↑spleen weights - both agents

Comment:

- NESP-[redacted] is a [redacted] form of NESP thought to be a potential manufacturing impurity. The sponsor conducted this study to address this potential concern. However, analysis of NESP-[redacted] was not performed with a validated method, not conducted under GLPs, thus the sponsor considered this study invalid. However, the [redacted] is no longer considered to be a major impurity.

18. NESP: Consideration of Need for Carcinogenicity Testing

The sponsor provided a written consideration regarding the lack of a need to perform a carcinogenicity study with NESP, as per the request of this reviewer. The paper was composed by [redacted]
The following is a summation of this paper:

NESP is a modified form of rhuEPO, which has been clinically administered in thousands of CRF patients for many years. This modified EPO [in AA sequence & additional carbohydrate chains] has been shown to be negative in genotoxic tests performed both via in vitro bacterial & mammalian assays, as well as using in vivo mammalian tests [in which the BM was the target organ].

NESP has been shown to bind only to EPO receptors and was detected only in BM when evaluated with various cryopreserved human tissues (as was EPO). This is further supported in the data generated from the 6-month rat & dog studies, in which there was no evidence of increased mitotic activity or hyperplasia noted upon microscopic evaluation of numerous tissues, with the expected exception of the BM [increased erythropoiesis].

The concern of the potential stimulation of pre-existing tumors is also an issue. However, this is not the endpoint of traditional carcinogenicity (bioassay) studies. In addition, there are no known EPO-responsive tumors. There are no neoplasms associated with renal failure in humans. There is the potential for cancer resulting from the enhanced proliferation of erythropoietic cells for either NESP or EPO. However, this neoplasia has not been evidenced with EPO in animals or humans. In fact, the intent of use of NESP or EPO is essentially as a replacement therapy

Reproduction/Teratology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Study of Fertility & Early Embryonic Development to Implantation in the Rat with NM321 via IV Injection; study #970001; performed (per GLP) at [redacted] lot [redacted] 11/98; part III of V, vol 30

2. Study of Fertility & Early Embryonic Development to Implantation in the Rat with NM321 via IV Injection; study #970141; performed (per GLP) at [redacted] lot [redacted] 5/99; part III of V, vol 31

3. Study of Fertility & Early Embryonic Development to Implantation in the Rat with NM321 via IV Injection; study #100136; performed (per GLP) at [redacted] lot [redacted], 6/99; part III of V, vol 32

4. A Combined Range-Finding Developmental Toxicity & TK/Placental Transfer Study in Rats with NM321 when Administered IV; study #960124; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 33

5. A Developmental Toxicity Study with NM321 in the Rat via IV Injection; study #970024; performed at [redacted] (per GLP); lot [redacted] 7/98; part III of V, vol 34

6. A Range-Finding Developmental Toxicity Study of NM321 in the Rabbit via IV Injection; study #970009; performed at [redacted] (per GLP); lot [redacted] 11/97; part III of V, vol 35

7. A Developmental Toxicity Study with NM321 in the Rabbit via IV Injection; study #970023; performed at [redacted] (per GLP); lot [redacted] 7/98; part III of V, vol 36

8. Study Effects on Pre- and Postnatal Development Including Maternal Functions in Rats with NM321 via IV Injection; study #970075; performed at [redacted] (per GLP); lot [redacted] 7/99; part III of V, vol 37

Reproduction/Teratology Studies

1. Study of Fertility & Early Embryonic Development to Implantation in the Rat with NM321 via IV Injection

Species: [redacted] rats (26/sex/grp)

Dose Level: 0, 0.5, 2.5, 10 µg/kg/dose

Route/Duration: IV

Males - dosed tiw for 4 wks premating, tiw during mating [21 days] & postmating - killed 1 wk postmating

Females - dosed tiw for 2 wks premating, through mating confirmation; dosed on GD 1, 3, 5, 7 - killed on GD 15

Methods:

Females - Clinical signs, BWs, food consumption, vaginal smears, pregnancy rate, uterine contents, ovary evaluation, gross evaluation

Males - Clinical signs, BWs, food consumption, gross evaluation, testicular/epididymal wts, sperm evaluation

Findings:

Males - 2/26 deaths - 10 µg/kg - wk 6/8
No other adverse effects on clin signs, BWs, food consumption

Enlarged spleens - 13/26 rats (2.5 µg/kg); 25/26 rats (10 µg/kg)
Discolored testes (red) - all grps

Testicular wts - comparable between grps, but 20-35% lighter compared to historical data

↓ sperm motility/sperm count - all grps

**An evaluation of 14 additional male rats from the supplier [redacted] revealed degeneration/atrophy in both testes of 8/14 rats. Necrosis of the germinal epithelium noted in 4/8 rats; oligospermia was noted in 6/14 rats; degenerated seminal products were noted in tubular lumens of 8/14 rats

Females - No adverse effects on clin signs, BWs, food consumption

Mating indices= 100%/96.2%/88.5%/96.2% at 0/0.5/2.5/10 µg/kg
Pregnancy rates = 61.5%/56.0%/47.8%/64.0% at 0/0.5/2.5/10 µg/kg

Mean preimplantation loss per rat = 1.1/2.3/3.2/2.6 at 0/0.5/2.5/10 µg/kg

Mean postimplantation loss/early resorptions per rat = 0.5/3.6/1.9/4.1 at 0/0.5/2.5/10 µg/kg

Mean number of live fetuses per rat = 13.8/9.6/8.3/7.9 at 0/0.5/2.5/10 µg/kg

Enlarged spleens - 13/26 rats - 10 µg/kg

Due to the apparent impaired fertility of the male rats provided by the supplier, the NOAEL could not be determined. The sponsor repeated the study [#970141]

2. Study of Fertility & Early Embryonic Development to Implantation in the Rat with NM321 via IV Injection
Species: [redacted] rats (26/sex/grp)
Dose Level: 0, 0.5, 2.5, 10 µg/kg/dose
Route/Duration: IV
Males - dosed tiw for 4 wks premating, tiw during mating [21 days] & postmating - killed 1 wk postmating

Females - dosed tiw for 2 wks premating, through mating confirmation; dosed on GD 1, 3, 5, 7 - killed on GD 15

Methods:

Females - Clinical signs, BWs, food consumption, vaginal smears, pregnancy rate, uterine contents, ovary evaluation, gross evaluation

Males - Clinical signs, BWs, food consumption, gross evaluation, testicular/epididymal wts, sperm evaluation

Findings:

Males -2/26 deaths- 2.5 µg/kg- wk 8/9; 2/26 deaths- 10 µg/kg- wk 7/8 Pale incisors - 16/25 - 10 µg/kg - wk 8/9

↓ BWs (5-7% lower than controls) - 10 µg/kg - premating until term

↓ food consumption - ≥2.5 µg/kg - premating

Enlarged spleens - 6/26 rats (2.5 µg/kg); 24/26 rats (10 µg/kg)
Discolored testes (red) - Rx grps

Females - Pale incisors - 10 µg/kg - wk 6
No other adverse effects on clin signs, BWs, food consumption

Mating indices= 100% - all grps
Pregnancy rates = 100%/88.5%/92.3%/88.5% at 0/0.5/2.5/10 µg/kg

Mean preimplantation loss per rat = 2.1/1.9/2.5/2.7 at 0/0.5/2.5/10 µg/kg

Mean postimplantation loss/early resorptions per rat = 0.8/3.3/3.7/6.7 at 0/0.5/2.5/10 µg/kg

Mean number of live fetuses per rat = 13.1/11.0/9.8/6.7 at 0/0.5/2.5/10 µg/kg

Comment:

- The report postulates that the increased levels of RBCs results in increased blood viscosity, which can lead to vascular congestion, blood stasis, & impaired blood flow, causing an O_2/nutrient deficiency to crucial tissues in the developing fetus.

The NOAEL was 0.5 µg/kg/dose for parental toxicity & 10 µg/kg/dose for fertility & reproductive performance. However, no NOEL could be established for embryotoxicity, due to the finding of increased postimplantation loss in all Rx grps.

3. Study of Fertility & Early Embryonic Development to Implantation in the Rat with NM321 via IV Injection

Species: [redacted] rats (26/sex/grp)

Dose Level: 0, 0.02, 0.1, 0.5 µg/kg/dose

Route/Duration: IV

Males - dosed tiw for 4 wks premating, tiw during mating [21 days] & postmating - killed 1 wk postmating

Females - dosed tiw for 2 wks premating, through mating confirmation; dosed on GD 1, 3, 5, 7 - killed on GD 15

Methods:

Females - Clinical signs, BWs, food consumption, vaginal smears, pregnancy rate, uterine contents, ovary evaluation, gross evaluation

Males - Clinical signs, BWs, food consumption, gross evaluation, testicular/epididymal wts, sperm evaluation

Findings:

Males - No adverse effects on clin signs, BWs, food consumption

Enlarged spleens - 6/26 rats (2.5 µg/kg); 24/26 rats (10 µg/kg)
Discolored testes (red) - Rx grps

Females - No adverse effects on clin signs, BWs, food consumption
Mating indices= 100% - all grps
Pregnancy rates = 88.5%/100%/88.5%/92.3% at 0/0.02/0.1/0.5 µg/kg

Mean preimplantation loss per rat = 2.7/2.2/1.5/2.0 at 0/0.02/0.1/0.5 µg/kg

Mean postimplantation loss/early resorptions per rat = 1.4/1.5/0.9/1.8 at 0/0.02/0.1/0.5 µg/kg

Mean number of live fetuses per rat = 13.1/15.0/16.3/14.6 at 0/0.02/0.1/0.5 µg/kg

The NOEL was 0.5 µg/kg/dose for parental toxicity; for fertility & reproductive performance; & for embryotoxicity.

4. A Combined Range-Finding Developmental Toxicity & TK/Placental Transfer Study in Rats with NM321 When Administered IV

Species: [redacted] rats (8 females/grp for range-finding study [RF]; 16 females/grp for the TK study)
Dose Levels: 0, 1, 5, 20 µg/kg/day (RF); 20 µg/kg/day (TK)
Route/Duration: IV injection
GD 5-16 (RF); GD 5-19, GD 11-19, GD 19 (TK)
Kills: GD 19 (RF & TK)

Methods: Clinical signs, BWs, food consumption, TK profile (sera, amniotic fluid, & fetal blood), anti-NM321 Abs, gross evaluation, and uterine data (RF & TK)

Findings: No mortality occurred; no Abs developed; pregnancy rates = 100% for all grps; no abnormalities in uterine implantation data & corpora lutea; fetal external exams
Enlarged spleen noted - 5/8 (5 µg/kg) & 8/8 (20 µg/kg)

RF - ↓food consumption - 20 µg/kg - GD 12-18
↓fetal BWs (11%) - 20 µg/kg [considered secondary to maternal toxicity, as no apparent placental transfer of NM321 occurred]

TK -(via [redacted] - levels of NM321 were quantifiable on GD 5,11,19 [limit of detection = 0.17 & 0.35 ng/mL]
Accumulation ratio = 1.40/0.94 for 9/15 daily doses

Fetal plasma levels were very low, with the maximum level (0.31 ng/mL) at 2-fold the limit of quantitation [0.166 & 0.112 ng/mL] - indicative of minimal placental transfer of NESP

Comment:

- Based on this study, the sponsor determined doses of 0, 1, 5, or 20 µg/kg/day would be used in study #970024.

5. A Developmental Toxicity Study with NM321 in the Rat via IV Injection

Species: [redacted] time-mated rats (24 females/grp)
Dose Levels: 0, 1, 5, 20 µg/kg/day
Route/Duration: IV injection; GD 6-15; C-sections on GD 20

Methods: Clinical signs, BWs, food consumption, hematology (GD 20), gross evaluation, uterine data, fetal evaluations

Findings:

Females - ↓BW gain - 20 µg/kg - GD 12-15
↓food consumption - ≥5 µg/kg - GD 12-15

No Rx-related abnormalities in uterine implantation data & corpora lutea

↑HCT, RBCs - Rx females
Enlarged spleens - 12/24 (20 µg/kg)

F_1 - ↓BWs - Rx grps - dose-related - 5-7% at 1 µg/kg & 10% at 20 µg/kg [considered to be possibly secondary to ↑ viscosity of maternal blood] - all data were within the historical ranges for the lab

No Rx-related external, visceral, skeletal variations/ malformations

The NOAEL was 1 µg/kg/day for maternal toxicity & fetal developmental toxicity.

6. A Range-Finding Developmental Toxicity Study of NM321 in the Rabbit via IV Injection
Species: [redacted] mated rabbits (6 females/grp)
Dose Levels: 0, 0.1, 2, 10, 20 µg/kg/day
Route/Duration: IV injection; GD 6-18; C-sections on GD 30

Methods: Clinical signs, BWs, food consumption, TK profile (sera), gross evaluation, uterine data, fetal evaluations

Findings:

Females - decreased feces - ≥10 µg/kg (starting ~GD 15)

↓BWs - 20 µg/kg - GD 6-30
↓food consumption - ≥10 µg/kg - GD 6-30

All of the Rx rabbits had quantifiable levels of NM321 on GD 18; terminal half-life increased w/ dose, due to faster clearance at low doses [7 hrs at 0.1 µg/kg to 20 hrs at 20 µg/kg]

No Rx-related abnormalities in uterine implantation data & corpora lutea

Enlarged spleens - 2/6 (20 µg/kg)

$\underline{F_1}$ - One fetus at 10 µg/kg - open eyes, exencephaly, cleft palate, flexure, indentation under lower jaw, kinked tail

Comment:

- Based on this study, the sponsor determined doses of 0, 1, 5, or 20 µg/kg/day would be used in study #970023.

7. A Developmental Toxicity Study with NM321 in the Rabbit via IV Injection

Species: [redacted] rabbits (20 females/grp)

Dose Levels: 0, 1, 5, 20 µg/kg/day

Route/Duration: IV injection; GD 6-18; C-sections on GD 30

Methods: Clinical signs, BWs, food consumption, hematology (GD 19), gross evaluation, uterine data, fetal evaluations

Findings:

$\underline{\text{Females}}$ - Sacrifice of 1/20 controls (GD 24) due to aborting; 1/20 (20 µg/kg) on GD 29 following early delivery

↓BWs - Rx grps - GD 6-30

↓food consumption (slight) - Rx grps - GD 6-30

No Rx-related abnormalities in uterine implantation data & corpora lutea

↑HCT, RBCs - Rx grps
Enlarged spleens - 1/20 (1 & 20 µg/kg)

$\underline{F_1}$ - ↓BWs - Rx grps (7-10% lower than controls) - all data were within the historical ranges for the lab

No Rx-related external, visceral, skeletal variations/ malformations

The NOAEL was 1 µg/kg/day for fetal developmental toxicity.

8. Study Effects on Pre- and Postnatal Development Including Maternal Functions in Rats with NM321 via IV Injection

Species: [redacted] rats (25 females/grp)

Dose Levels: 0, 0.5, 2.5, 10 µg/kg/dose, every other day [dosing regimen based on long half-life (14 hrs) in rats]

Route/Duration: IV injection; GD 6-20 & LD 1-23

Kills: F_0 - LD 24
F_1 - some culled on LD 4; some killed on LD 24; remaining allowed to deliver the F_2 pups & killed on LD 4
F_2 - LD 4

Methods:
F_0 - Clinical signs, BWs, food consumption, gross evaluation
F_1 pups - survival, growth, & development [eye opening, pinna detachment, auditory startle, vaginal patency, preputial separation], gross evaluation
(25 pups/sex/grp) - open field, Biel multiple T-water maze; followed by mating at 70-90 days old

F_2 pups - appearance, growth, survival, gross evaluation

Findings:

$\underline{F_0}$ - Death - 1/25 (2.5 µg/kg) - LD 21
Swollen paws/limbs - ≥2.5 µg/kg - during lactation
Irregular gait - ≥2.5 µg/kg - during lactation (seen mainly in females with swollen paws)

Emaciation - 2/25 (10 µg/kg) - during lactation
↓ food consumption - ≥2.5 µg/kg - during lactation

Livers - dark red - Rx grps
Spleen - enlarged - ≥2.5 µg/kg

$\underline{F_1}$ Pups - Deaths/euthanasia - 0.5 µg/kg (1 %); 2.5 µg/kg (2 males); 10 µg/kg (2 males) - 3-6 wks postweaning - attributed to poor physical condition

Sex distribution, live/dead pups - no differences
Pup survival [LD 1-4] - 99.3% (control), 97.7% (0.5 µg/kg), 98.0%(2.5 µg/kg), 97.6% (10 µg/kg)
Pup survival [LD 5-24] - 99.2% (control), 99.6% (0.5 µg/kg), 99.2% (2.5 µg/kg), 93.5% (10 µg/kg)

↓ BWs - Rx grps - LD 1 (7-10%)
↓ BWs - LD 24 (2.5 µg/kg - 18% & 10 µg/kg - 37%)

No effect on pinna detachment, auditory startle, open field, water maze

Eye opening - mean age of 14.4 days (control), 14.7 days (#2.5 µg/kg), 15.2 days (10 µg/kg) - reflective of ↓ BWs

Vaginal Patency - mean age of 32/31/33/33 days for 0/0.5/2.5/10 µg/kg - reflective of ↓ BWs

Preputial Separation - mean age of 44/45/45/47 days for 0/0.5/2.5/10 µg/kg - reflective of ↓ BWs
Liver - Pale - 10 µg/kg - LD 24

F_1 Parents - ↓BWs - wks 7-14 - ≥2.5 µg/kg [premating]
↓BWs - females - ≥2.5 µg/kg - during gestation/lactation interval

Mating - male fertility indices - 100% (control), 95.7% (0.5 µg/kg), 91.3% (2.5 µg/kg), 87% (10 µg/kg)
[Controls - historical range = 82.8-100%]

Pregnancy rates - 100% (control), 95.8% (0.5 µg/kg), 92% (2.5 µg/kg), 84% (10 µg/kg)
[Controls - historical range = 82.8-100%]

F_2 Pups - No effect on sex distribution, live birth indices, survival, BWs, external evaluation (to LD 4)

The NOAEL was 0.5 µg/kg/dose for peri/postnatal developmental toxicity.

Mutagenicity Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Bacterial Reverse Mutation Assay with an Independent Repeat Assay; study #960031; performed at [redacted] (per GLP); lot [redacted]; 9/96; part III of V, vol 39

2. In Vitro Mammalian Cell Gene Mutation Test with NM321; study #960134; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 39

3. Mammalian Erythrocyte Micronucleus Test with NM321; study #960133; performed at [redacted] (per GLP); lot [redacted] 4/97; part III of V, vol 39

Mutagenicity Studies

In Vitro Bacterial System

1. Bacterial Reverse Mutation Assay with an Independent Repeat Assay

NM321 at levels of 100, 333, 1000, 3333, or 5000 µg/plate were incubated with [redacted] in the presence & absence of [redacted]. NM321 did not cause a positive response [increased number of revertants] for any of the tester strains.

Mammalian System

2. In Vitro Mammalian Cell Gene Mutation Test with NM321

NM321 at levels of 1.5, 15, 50, 150, or 374 µg/mL were incubated with CHO cells in the presence & absence of [redacted]. NM321 did not cause a positive response [increased number of forward mutations at the [redacted] locus].

3. Mammalian Erythrocyte Micronucleus Test with NM321

Male mice were IP injected at 37.5, 120, or 375 µg/kg of NM321, followed by kills at 24 & 48 hrs. Microscopic examination of BM cells revealed reductions (up to 26%) in the ratio of poly-chromatic RBCs/total RBCs [suggestive of bioavailability of NM321 to the BM]. No significant increase in micronucleated poly-chromatic RBCs (i.e., clastogenic potential) was noted.

Safety Pharmacology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Test for Antagonism to Acetylcholine, Histamine, & Barium Chloride in the Isolated Guinea Pig Ileum Exposed to NM321; study #970041; performed by [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 41

2. A Cardiovascular Toxicity Study of NM321 Administered via IV Injection to Dogs; study #963607; performed at [redacted] (per GLP); lot [redacted] 7/97; part III of V, vol 41

3. Airway Resistance & Dynamic Lung Compliance Assay in Guinea Pigs Administered NM321 via IV Injection; study #970037; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 42

4. Neuropharmacological Profile in Mice Administered NM321 via SC Injection; study #970038; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 42

5. Phenylquinone (PPQ) Writhing Assay in Mice Administered NM321 via SC Injection; study #970039; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 42

6. Determination of Electrolyte Concentrations & Volume Diuresis in Rats Administered NM321 via SC Injection; study #970040; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 42

7. Evaluation of Chemically-Induced Seizures (Pentylenetetrazol or Strychnine) in Mice Administered NM321 via SC Injection; study #970042; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 42

8. Submaximal Electroshock Seizure Assay in Mice Administered NM321 via SC Injection; study #970043; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 42

9. Antipyretic Evaluation Assay in Rats Administered NM321 via SC Injection; study #970044; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 43

10. Gastrointestinal Propulsion Assay in Mice Administered NM321 via SC Injection; study #970045; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 43

11. Barbiturate Sleep Time Potentiation Assay in Mice Administered NM321 via SC Injection; study #970046; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 43

12. Spontaneous Motor Activity (SMA) Assay in Mice Administered NM321 via SC Injection; study #970047; performed at [redacted] (per GLP); lot [redacted] 1/98; part III of V, vol 43

Safety Pharmacology Studies

1. Test for Antagonism to Acetylcholine, Histamine, & Barium Chloride in the Isolated Guinea Pig Ileum Exposed to NM321

Species: [redacted] guinea pigs (2 males) - 4 tissues per agonist (acetylcholine, histamine, and barium chloride) & 12 tissues per CSF dose

Dose Level: 0, 10 µg/mL

Methods:

1. Ileum sections were exposed to an agonist at a concentration to cause a vigorous but submaximal contraction (determined by a [redacted]. These data served as reference data.
2. Ileum was exposed to NESP, followed by addition of an agonist. The change in contraction intensity was measured.

Findings:

The mean observed responses of isolated ileum to the various agonists were not significantly antagonized by exposure to NESP.

2. A Cardiovascular Toxicity Study of NM321 Administered via IV Injection to Dogs

Species: [redacted] dogs (1/sex/grp)

Dose Levels: 1, 20, 50, 100 µg/kg/dose

Route/Duration: IV injection (via surgically implanted catheter in the vena cava)/single dose + kill on day 8

Methods: Clinical signs, BWs, food consumption, respiration rate, cardiovascular parameters, oxygen saturation, clinical pathology (baseline & day 6/8) were performed

Findings:

No abnormalities in cardiovascular or respiratory parameters or in ECGs.

↓ BWs - ≥20 µg/kg - postdose interval
↑ RBCs, HCT, HGB, retics - all grps
↓ WBCs - all grps

3. Airway Resistance & Dynamic Lung Compliance Assay in Guinea Pigs Administered NM321 via IV Injection

Species: [redacted] GPs (4 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: IV injection/single dose + kill on day 1

Methods: GPs were anesthetized with urethane, instrumented, & positioned inside a single chamber plethysmograph prior to, and during dosing. Responses were evaluated every minutes for the first 5 mins, followed by every 5 mins for 30 mins.

Findings: Dynamic lung compliance & airway resistance were not adversely affected for any Rx grp.

4. Neuropharmacological Profile in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 2

Methods: Following dosing, the mice were placed within a wooden square covered with a mesh lid, on top of absorbent paper, & observed at 15, 30, 45 mins & 1, 2, 3, 4, & 24 hrs postdose. Body temps were measured at 1 hr postdose.

Findings: No adverse effects were noted.

5. Phenylquinone (PPQ) Writhing Assay in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: One hr postdose, the mice were challenged with PPQ (0.02%) injection (IP route), & observed for 10 mins [total number of writhes recorded].

Findings: A 17%, 10%, & 4% inhibition in the mean number of writhes occurred for 4.5, 13.5, & 45 µg/kg.

6. Determination of Electrolyte Concentrations & Volume Diuresis in Rats Administered NM321 via SC Injection

Species: [redacted] rats (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: Urine was collected from fasted rats for 4 hrs postdose; volumes, pH, Na+, K+, & Cl- determined.

Findings: No significant changes in the parameters evaluated were noted; no apparent diuretic effect occurred.

7. Evaluation of Chemically-Induced Seizures (Pentylenetetrazol or Strychnine) in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: About 60 mins postdose, pentylenetetrazol (125 mg/kg) or strychnine (1.5 mg/kg) was administered [SC injection], followed by observation for 60 mins.

Findings: No anticonvulsant effect was noted in the NESP-Rx mice

8. Submaximal Electroshock Seizure Assay in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: Mice were individually placed in plexiglass squares, followed by dosing with NESP. About 60 mins postdose, electroshock intensities of 5, 8, or 10 mA were administered transcoronally with saline-moistened electrodes, followed by a 1-min observation period.

Findings: Tonic extensions of 0% (5 mA), 30% (8 mA) & 50% (10 mA) were noted. NESP neither potentiated nor inhibited the electroshock effect, as compared to controls.

9. Antipyretic Evaluation Assay in Rats Administered NM321 via SC Injection

Species: [redacted] rats (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 2

Methods: Rats were SC injected with a 15% yeast suspension, followed (24 hrs later) with injection of NESP. Body temps were recorded at 1 hr (25 hrs post-yeast injection).

Findings:

Body temps [°C] - pre-yeast - 37.23 (control); 37.33 (4.5 µg/kg); 37.28 (13.5 µg/kg); 37.13 (45 µg/kg)

Body temps [°C] - 23 hrs - 38.90 (control); 38.88 (4.5 µg/kg); 39.05 (13.5 µg/kg); 38.83 (45 µg/kg)

Body temps [°C] - 25 hrs - 38.88 (control); 38.83 (4.5 µg/kg); 38.98 (13.5 µg/kg); 39.10 (45 µg/kg)

The injection of NESP did not result in an antipyretic effect.

10. Gastrointestinal Propulsion Assay in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: A 10% suspension of activated charcoal USP (10 mL/kg), was orally administered 30 minutes after NESP injection. The mice were killed 30 minutes later and the distance traveled by the charcoal in the intestines was measured.

Findings: Gastric motility was inhibited by 8%, 9%, 20% for 4.5, 13.5, & 45 µg/kg NESP respectively - not a significant effect on GI peristalsis.

11. Barbiturate Sleep Time Potentiation Assay in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: An IP injection of sodium pentobarbital (50 mg/kg) was given 1 hr after NESP injection, & the difference between the time of loss of the righting reflex & the time at which the righting reflex was regained, was determined.

Findings: Increases of 43% (4.5 µg/kg), 50% (13.5 µg/kg), & 49% (45 µg/kg) in sleep time were noted.

12. Spontaneous Motor Activity (SMA) Assay in Mice Administered NM321 via SC Injection

Species: [redacted] mice (10 males/grp)

Dose Levels: 0, 4.5, 13.5, 45 µg/kg/dose

Route/Duration: SC injection/single dose + kill on day 1

Methods: At 30 mins following dosing of NESP, mice were put into a computerized photobeam activity system [for 20 mins] in order to record spontaneous motor activity [by breaking the light beam].

Findings: No significant changes in SMA was noted in mice Rx with NESP, compared to controls.

CONCLUSION:

The proposed clinical indication [per the package insert] for NESP is for the treatment of anemia associated with CRF, as NESP has been shown to stimulate erythropoiesis in this patient population. This treatment has been correlated with a reduction in RBC transfusions and improved quality of life.

NESP is a well-characterized recombinant protein, which is an analog of recombinant human erythropoietin (EPO). NESP stimulates erythropoiesis in a manner identical to endogenous human EPO, as well as recombinant human EPO - which is approved for use in the Rx of anemia in patients with chronic renal failure (CRF). NESP binds to cell surface receptors that are specific for EPO, resulting in the promotion of survival, proliferation, & differentiation of cells of the erythroid lineage. The in vitro potency of NESP is lower than that of EPO due to an increase in negative charge resulting from the addition of [redacted] containing carbohydrate & consequently weaker receptor binding. However, due to the addition of this carbohydrate, the in vivo activity of NESP is increased relative to EPO because of its longer serum half-life [~3-fold longer].

Activity studies performed in rodents and dogs showed that NESP injection resulted in a dose-dependent increase in red cell mass production.

Preclinical studies were performed in rats and dogs, which are species in which NESP is pharmacologically active. The sponsor used either the SC or IV route, as either route is indicated for humans. In addition, the dosing regimen in the animals attempted to mimic the proposed human schedule - qiw or tiw. All preclinical studies were performed using the NESP formulation

containing HSA. An additional PK study was performed in dogs, as well as a PK crossover study in humans using the HSA-free NESP formulation.

Single IV injections in rats and dogs showed expected exaggerated pharmacological effects of NESP at levels of ≥0.135 µg/kg, consisting of increased red cell mass, with a compensatory reduction of PLT production. Increased reticulocyte levels were noted for both species and serum iron levels were decreased in dogs [due to the rapid RBC production]. Although a NOEL was not achieved due to the exaggerated pharmacological response seen, NESP was well tolerated at doses up to 150 µg/kg (dogs) and 200 µg/kg (rats).

Daily injections (SC or IV routes) in rats for 14 days at doses ranging from ~0.135 to 13.5 µg/kg/day, resulted in findings similar to the single dose studies.

Rats and dogs were SC or IV injected tiw, for 4 weeks at NESP doses ranging from 1 to 100 µg/kg/dose. Findings included deaths; decreased body weights; red/thickened ears (rats); increased red cell mass, neutrophils, and reticulocytes; decreased PLTs, MCH, MCHC, and iron [reflective of the rapid incorporation of iron into the HGB of the new RBCs]; increased liver transaminases; decreased glucose, cholesterol, protein (rats); extramedullary hematopoiesis in the spleen and liver; congestion in various major organs [due to the polycythemia]; bone marrow hypercellularity; myelofibrosis (dogs); and injection site reactions. A NOEL could not be achieved in these studies due to the exaggerated pharmacological responses observed at all dose levels.

Rats and dogs were also IV injected qiw, for 3 months at NESP doses ranging from 1.5 to 30 µg/kg/dose and tiw for 3 months at doses of 0.5 µg/kg/dose. The findings were similar to those exhibited in the 4-week studies. Additional observations included retinal and choroidal congestion (rats); injection of scleral blood vessels (due to elevated RBCs in dogs); anaphylactic reactions (due to HSA content in dogs); gastric erosions (rats); focal myocardial degeneration (rats); and kidney necrosis/tubular degeneration. The NOAEL in rats was 1.5 µg/kg/dose, qiw, or 0.5 µg/kg/dose, tiw. The NOAEL in dogs was 1.5 µg/kg/dose, qiw. Again, a NOEL could not be achieved in these studies due to the exaggerated pharmacological responses observed at all dose levels.

Rats and dogs were used for 6-month IV toxicity studies of NESP, initially intended to evaluate a tiw regimen at 1 to 20 μg/kg/dose. The tiw dose levels for all groups had to be lowered to 0.5 to 10 μg/kg/dose for both species due to the toxicities noted, followed by further adjustment to a qiw regimen to 1.5 to 30 μg/kg/dose. Many of the findings were again similar to those noted in the earlier studies.

The pathologic changes to various tissues [i.e., congestion, fibrosis, thrombosis of the heart] was generally reflective of the high viscosity of the blood as a result of NESP administration, which led to vascular stress. The CNS toxicities seen in the dogs (seizures, ataxia, etc...) were thought to be due to blood clots. These long-term studies resulted in more extensive pathological alterations to various tissues. Of note were the kidney changes (tubular nephropathy and infarcts), heart findings (intimal hyperplasia, degenerated myofibers) in the rats.

The development of anti-NESP antibodies occurred in some rats and dogs, however there was no resultant change in the PK profile or in other toxicology parameters (i.e., neutralization). Mice that were SC injected with EPO or NESP displayed dose-related increases in the number of mice that developed antibodies.

Evaluation of the distribution of NESP in nephrectomized rats revealed a negligible renal contribution to the overall clearance of NESP (up to 2.4% excreted). The proposed clearance mechanism of NESP is desialylation by tissue and blood sialidases, followed by hepatic removal, as evidenced when desialylated NESP was rapidly cleared from the circulation via the hepatic galactose receptor. The PK profiles of two NESP formulations - one HSA-containing and one HSA-free - were similar when IV or SC injected into dogs. Another study showed that the clearance of NESP was inversely related to the sialic acid content [the greater the amount of sialic acid, the larger was the molecular weight of the NESP mixture].
Note that studies performed with rhuEPO have shown effects similar to those noted in the various toxicity studies performed using NESP. These include:

- ↑RBCs, HCT, HGB, retics, PT, APTT; ↓MCV, MCH, MCHC, M/E ratio

- ↑ALT, LDH, bilirubin, electrolytes, CPK, total protein; ↓glucose, cholesterol, urine specific gravity [due to prolonged polycythemia & increased erythropoiesis]

- Polycythemia, followed by anemia in dogs dosed SC/IV up to 520 IU/kg/day for 90 days - possibly due to Ab development, receptor down-regulation, frequent phlebotomy

- Myelofibrosis in dogs receiving 2000 U/kg/day for 4 weeks or 100 U/kg/day for one year and in rats at 50 or 250 U/kg/day for one year

- Extramedullary erythropoiesis (spleen); gastric erosions (rats up to 2000 IU/kg/day for 4 weeks & up to 500 IU/kg/day for 13 weeks, IV injections); fibrosis in the Bowman's capsule in dogs dosed up to 500 IU/kg/day for one year

- Redness of pinnae & limbs in rats at 400-2000 IU/kg/day for 4 weeks and at 100-500 IU/kg/day for 13 weeks; dilation of vessels of the eye in rats (100-500 IU/kg/day for 13 weeks) & monkeys (500-1000 IU/kg/day for 13 weeks)

- Anaphylactic reactions [dyspnea, ataxia, salivation, emesis, pale mucous membranes, swollen lips] in dogs at 2800 IU/kg/dose, tiw, for 3 weeks

- Conjunctival injection & redness of oral mucosa in dogs at 200-2000 IU/kg/day for 4 weeks

- Bloody/mucoid feces, presence of urine occult blood in dogs at 20-500 IU/kg/day for 13 weeks

- BM fibrosis in dogs at 500 IU/kg/day for 13 weeks - present to a lesser extent after 5-week recovery; in dogs at 100-500 IU/kg/day for 52 weeks

- Deaths/moribund kills in 50% of dogs at 100 IU/kg/day & 60% at 500 IU/kg/day (52-weeks), with prostration, tremors, dyspnea

- Swollen kidneys, with congestion & glomerular dilation in dogs at 100-500 IU/kg/day for 52 weeks

[Note that 1 Unit of EPO = 5.050 ng peptide mass]

Administration of NESP via IV injection to rats from prior to mating to approximately gestation day 7 (Segment I) revealed a NOAEL of 0.5 μg/kg/dose for F_0 parental toxicity (deaths and reduced body weights) and 10 μg/kg/dose for fertility and reproductive function of the F_0 animals. The NOEL for embryotoxicity (postimplantation loss) was 0.5 μg/kg/dose, believed to occur as a result of impaired blood flow across the placenta due to the increased blood viscosity.

In addition, the IV injection of NESP in pregnant rats and rabbits during gestation (Segment II) resulted in a NOAEL of 1 µg/kg/day for both dams and fetuses. The dams displayed the expected pharmacological response to NESP [i.e., decreased body weights, increased HCT and RBCs, and enlarged spleen]. The F_1 fetuses exhibited reduced body weights, likely secondary to the maternal outcome. No teratogenic concerns surfaced at NESP doses up to 20 µg/kg/day. A NOAEL of 0.5 µg/kg/dose was achieved with IV injection of NESP into pregnant F_1 rats in a perinatal/ postnatal study. A low incidence of the F_1 pups died at 3-6 wks postweaning, likely from a poor physical condition (i.e., decreased BWs). Higher doses of 2.5 and 5 µg/kg/dose resulted in decreased body weights and delayed development (eye opening and preputial separation). No adverse effects were noted with the F_2 generation (pups).

Safety pharmacology studies performed in mice, rats, and guinea pigs showed that administration of NESP did not adversely affect motor, neuromuscular, or autonomic functions, or GI motility. No adverse effects on the cardiovascular system were displayed by dogs that were IV injected with NESP. This agent was also found to be non-hemolytic when exposed to human blood, ex vivo.

No mutagenic potential was exhibited for NESP via in vitro or in vivo mammalian systems.

Following in vitro exposure to 23 different human tissues, both NESP and EPO bound only to bone marrow, which expresses EpoR. In an in vivo genotoxicity study in which the bone marrow is the target tissue, NESP was not mutagenic. In addition, in 6-month toxicology studies performed in rats and dogs, there was no evidence of increased mitotic activity or hyperplasia/ proliferation in any of the tissues examined, with the exception of the expected pharmacology of NESP. The theoretical concern that the enhanced proliferation of erythropoietic cells due to exposure to either NESP or EPO will result in cancer, has not been documented with use of rhEPO in animals or humans. Thus the sponsor is relying on the wealth of clinical data that have been generated with the use of rhEPO to serve as a replacement for the traditional rodent carcinogenicity study.

The preclinical data adequately support use of the product, ARANESP™, for the indication specified by the sponsor.

Mercedes A. Serabian, M.S., DABT, Toxicologist 6/23/03; revised 7/24/03

Key Words: EPO; NESP; recombinant human erythropoietin; glycosylation; chronic renal failure; RBC; HCT; anemia; myelofibrosis

MDK 7/24/03
concurrences:
OTRR/C,P-T/MGreen